

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



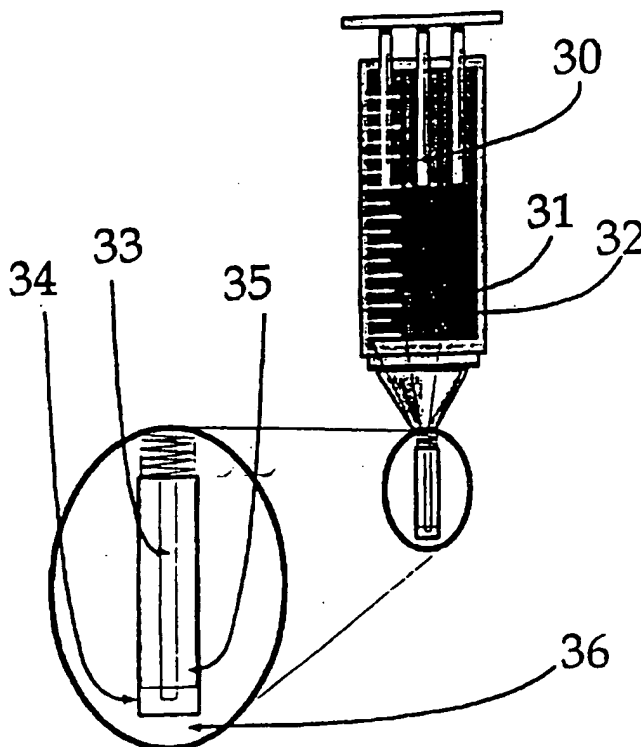
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/75, 14/745, A61K 9/10, B01D 15/00, 17/025, 17/038		A1	(11) International Publication Number: WO 96/17871
			(43) International Publication Date: 13 June 1996 (13.06.96)
(21) International Application Number: PCT/US95/15171		(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GR, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).	
(22) International Filing Date: 7 December 1995 (07.12.95)		Published With international search report.	
(30) Priority Data: 08/351,010 7 December 1994 (07.12.94) US			
(71) Applicant: PLASMASEAL CORPORATION (US/US); Suite 7, 1360 Montgomery Street, San Francisco, CA 94133 (US).			
(72) Inventors: ANTANAVICH, Richard, D.; 6830 Chardonnay Place, Paso Robles, CA 93446 (US); DORIAN, Randel; 15 Oak Road, Orinda, CA 94563 (US).			
(74) Agents: WALKER, William, B. et al.; Oblon, Spivak, McClelland, Maier & Neustadt, P.C., 4th floor, Crystal Square Five, 1755 Jefferson Davis Highway, Arlington, VA 22202 (US).			

(54) Title: PLASMA CONCENTRATE AND TISSUE SEALANT COMPOSITIONS

(57) Abstract

An inexpensive device, shown in the figure, for preparing tissue sealant is disclosed. The device is particularly applicable to preparation of autologous tissue sealant. A method of sealing tissue in which the tissue sealant is applied immediately after mixing platelet-rich plasma concentrate with a solution of calcium and thrombin is also disclosed. Adhesive and tensile strengths are comparable or superior to pooled blood fibrin sealants made with precipitation methods. Antifibrinolytic agents are not necessary because the tissue sealant contains high concentrations of natural inhibitors of fibrinolysis from the patient's blood. The tissue sealant also contains patient platelets and additional factors not present in available fibrin sealants that promote wound healing.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LJ	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

TITLE OF THE INVENTIONPLASMA CONCENTRATE AND
TISSUE SEALANT COMPOSITIONS

5

BACKGROUND OF THE INVENTIONField of the Invention:

10 The present invention concerns compositions, methods and
apparatuses for making concentrated plasma and tissue sealant,
for sealing tissue, for rapid separation of higher molecular
weight components in mixtures, for rapid separation of blood
plasma and for rapid separation of particulate components in
15 mixtures. The present invention is particularly applicable to
preparation and use of autologous tissue sealant.

Discussion of the Background:Fibrin Glues and Sealants

20 Various substances have been tried to meet the need for a
suitable tissue adhesive for use in surgical procedures.
Completely synthetic materials such as cyanoacrylate have been
tried and been found wanting. Natural fibrin glues and sealants
made from blood components were recognized early in the
development of this technology. Surgical "fibrin sealants"
25 (sometimes called "fibrin glues") made up of human fibrinogen
activated by bovine thrombin are used extensively in Europe.

-2-

Such fibrin sealants have been shown to be superior to synthetic adhesives or traditional surgery in many situations. In addition they reduce the need for blood transfusions. Academic and surgical opinion on fibrin sealants is very favorable. A recent review says:

Fibrin sealants are the most successful tissue adhesives to date. They have many advantages over adhesive technologies such as cyanoacrylates and marine adhesives in terms of biocompatibility, biodegradation and hemostasis. There are several commercial products in Europe but none in the United States due to the current regulatory stance against pooled plasma blood products (Sierra, 1993; vide infra).

In current practice, fibrin sealant is made by isolating a concentrate of human fibrinogen, fibronectin and factor XIII, usually by cryoprecipitation, and combining it immediately before use with bovine (or sometimes human) thrombin. The thrombin converts the fibrinogen to fibrin which rapidly gels to form a transient hemostatic plug, which is then stabilized by factor XIII. Fibronectin provides adhesion for cells repopulating the clot and tissue. The most common method of application of fibrin sealant is mixing of concentrated fibrinogen from pooled human blood with bovine thrombin and calcium immediately before use.

-3-

Fibrin sealant is not available in the U.S. commercially, but is in Europe (TISSEEL®, TISSUCOL®/Immuno; BERIPLAST®/Behring). Many papers have been published on its use.

5 Use of fibrin sealant in the United States is limited to preparation within the clinic outside FDA control. The reasons for FDA reluctance to approve these products in the United States are:

- virus transmission (e.g., HIV, hepatitis) from pooled human blood¹ and
- 10 • immunological reactions to bovine thrombin², for example thrombin and factor V inhibitors or foreign body reactions³.

These FDA concerns are so serious that the FDA has not approved any fibrin sealant product, despite strong interest from
15 surgeons and very favorable comparative studies in the literature.

These circumstances have led to much attention being given to methods to isolate an autologous counterpart of the fibrinogen containing component in the TISSUCOL system in a practical
20 manner. These efforts are discussed in the reviews cited below. The following from a review by Thompson⁴ shows that the value of stat autologous fibrinogen is much anticipated:

-4-

Fibrin glue is composed of two separate solutions of fibrinogen and thrombin. When mixed together, these agents mimic the last stages of the clotting cascade to form a fibrin clot. Fibrin glue is available in Europe but is not commercially available in the U.S.; therefore, investigators have extemporaneously compounded their own fibrin glue. Fibrinogen can be obtained from pooled, single-donor, and autologous blood donors and is usually isolated by the process of cryoprecipitation... The safest preparations use the patient's own blood to prepare fibrin glue... Use of... autologous blood results in minimal risk of disease transmission, but it requires anticipated use as in an elective procedure. The use of autologous blood usually is not possible in trauma or an emergency surgical procedure.

Siedentop' describes a number of approaches to the precipitation of fibrinogen from plasma in the context of the proposed use of this material as the fibrinogen furnishing component of a fibrin glue. Four methods were suggested: precipitation with ethanol, use of unfractionated plasma, cryoprecipitation, and precipitation with ammonium sulfate. Epstein' suggests the use of a fibrinogen preparation from autologous plasma obtained using polyethylene glycol precipitation. A system for preparing autologous tissue adhesive

-5-

using a relatively complex system based on ethanol precipitation has been described by Weis-Fogh⁷. Because none of these methods has yet produced a clearly superior autologous sealant, research in the past five years has continued on several approaches including plasma sealant⁸, ethanol precipitation⁹ and rapid cryoprecipitation¹⁰. Prior patents are discussed in later sections, Autologous Precipitate Sealants and Autologous Plasma Sealants.

None of these methods is readily adaptable for convenient use of an autologous plasma fraction as an adhesive which can be prepared quickly during the surgical procedure. All of the approaches suggested for preparation of the fibrinogen containing fraction for this purpose are too time-consuming and complex to be finished in a short enough time to be accomplished during the surgery. Also, in some procedures, such as cryoprecipitation, special equipment, such as refrigerated centrifuges, is required. While the prior art approach is to prepare the composition in advance, this immediately imposes the necessity for additional procedures for identification and retrieval of the samples matched with the patient, and the concomitant opportunity for error, besides the inconvenience to the patient, who must then arrange time for an additional medical appointment. And, of course, this practice is not possible when the surgery is conducted on an emergency basis.

-6-

Several useful reviews on surgical glues generally and on fibrin glues particularly have been published (Sierra, D. H. "Fibrin sealant adhesive systems: a review of their chemistry, material properties and clinical applications." J Biomater Appl 7 (4 1993): 309-52; Wiseman, David M., David T. Rovee, and Oscar M. Alvarez. "Wound Dressings: Design and Use." In Wound Healing: Biochemical & Clinical Aspects, ed. I. Kelman Cohen, Robert F. Diegelmann, and William J. Lindblad. 562-580. 1st ed., Vol. Philadelphia: W. B. Saunders Company, 1992). A review of devices used by surgeons discusses fibrin glue in context of both hemostatic agents and adhesive agents (Edlich, Richard F., George T. Rodeheaver, and John G. Thacker. "Surgical Devices in Wound Healing Management." In Wound Healing: Biochemical & Clinical Aspects, ed. I. Kelman Cohen, Robert F. Diegelmann, and William J. Lindblad. 581-600. 1st ed., Vol. Philadelphia: W. B. Saunders Company, 1992). A review of the role of fibrin and fibrinogen in blood coagulation has also been published (Jackson, C. M. and Y. Nemerson. "Blood coagulation." Annu Rev Biochem 49 (811 1980): 765-811). Other reviews, in reverse chronological order, include:

- (1) Lerner, R. and N. S. Binur. "Current status of surgical adhesives." J Surg Res 48 (2 1990): 165-81.

-7-

- (2) Gibble, J. W. and P. M. Ness. "Fibrin glue: the perfect operative sealant?" Transfusion 30 (8 1990): 741-7.
- (3) Thompson, D. F., N. A. Letassy, and G. D. Thompson. "Fibrin glue: a review of its preparation, efficacy, and adverse effects as a topical hemostat." Drug Intell Clin Pharm 22 (12 1988): 946-52.

Biological Precipitate Sealants

The vast majority of prior methods for preparing blood derived glues or sealants use fibrinogen precipitated (usually cryoprecipitated) from pooled plasma, a method first published in 1972¹¹. Most biological sealant patents are improvements on cryoprecipitation. Most go through a lyophilization step before application. A few touch on plasma, but none mentions a composition derived from concentrated plasma.

A composition from cryoprecipitation method was patented by Immuno AG¹². Since the basic method had been published previously, the composition claims were precisely descriptive:

Exemplary Claim: 1. A lyophilized tissue adhesive of mammalian protein origin which comprises fibrinogen, albumin, factor XIII, cold-insoluble globulin and plasminogen-activator inhibitor or plasmin inhibitor wherein

-8-

the fibrinogen is present in at least 33% by weight, the ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen is at least 80; and fibrinogen and albumin are present in a ratio of 33 to 90:5 to 40.

Several inventions have improved on early cryoprecipitation methods. A variation of cryoprecipitation is claimed to produce material superior for patients with blood coagulation disorders¹³. Stroetmann overcame problems by mixing fibrinogen, thrombin and protease inhibitor in lyophilized form for administration as a powder¹⁴. Behring found a way to improve solubility of (poorly soluble) cryoprecipitate¹⁵. French scientists have improved the traditional cryoprecipitation by using a double cold ethanol precipitation¹⁶. Epstein has a patent application for precipitation with polyethylene glycol (only the sister (device) patent has issued)¹⁷. Cryolife has crafted a patent with a broad exemplary claim¹⁸ (quoted in note), but all the claims are limited by use of a precipitation step.

Sierra's group has patented compositions with collagen added to fibrinogen. The notion is that collagen improves the mechanical properties of the sealant¹⁹. Related approaches are addition of hyaluronic acid to make the solution more viscous

- 9 -

before gelation (patent application)²⁰ and addition of silk fibroin for mechanical strength²¹.

A disclosed World patent application shows that Baxter is using fibrinogen affinity chromatography to get around the problems with precipitation²². A disclosed European patent application shows that Squibb scientists have developed a method eliminating the need for thrombin²³. They use fibrin made by passing fibrinogen through a column with immobilized snake-venom; chaotropic agents prevent gelation. According to this work, "(f)ibrin I monomer is preferred because it can, in contrast to fibrinogen, readily be converted to fibrin polymer without the use of thrombin or factor XIII." From blood, the method yields 60-90% fibrin monomer. It is activated by calcium and a pH change.

The majority of biological patents assume a precipitation step in the manufacture of the tissue sealant.

Autologous Precipitate Sealants

As discussed above, it is widely recognized that autologous blood products are superior for safety and biocompatibility reasons alone. Columbia University has patented the use of autologous fibrin glue²⁴, but the claims are restricted to cryoprecipitate. A European patent application makes similar claims²⁵, but again the claims are restricted to cryoprecipitate.

-10-

Autologous Plasma Sealants

Alterbaum has patented using small quantities of patient plasma to make tissue glue²⁶, but the plasma is not concentrated and the claims are restricted to use of flat pack centrifugation for isolation of the plasma.

1. A method for use in the autologous preparation of fibrin glue wherein a patient's blood is separated in a centrifuge having cylindrical cups pivotally mounted to a rotor to obtain plasma and wherein the plasma is separated in the centrifuge to produce concentrated fibrinogen, comprising the steps of: (a) transferring the blood or plasma into a substantially flat packet; (b) fixing the packet containing the blood or plasma in a recess of a substantially cylindrical insert fixture assembly; (c) inserting said cylindrical-shaped insert fixture assembly in a cup of the centrifuge having a complementary cylindrical shape so that the insert fixture assembly is held snugly within said centrifuge cup; (d) centrifuging the blood or plasma contained within said packet fixed in said insert fixture assembly held in said centrifuge cup to separate the blood or plasma into components; and ~~(e)~~ forming fibrin glue from one of said separated components.

-11-

Although no claim mentions or suggests concentrating the plasma, the patent which appears to be most similar to the present invention (Dennis Galanakis, SUNY Stony Brook) covers unconcentrated plasma glue²⁷:

5 1. A method of treating with autologous mammalian plasma fibrin to affect hemostasis, comprising the steps of:
10 (a) obtaining a sample of blood from said animal; (b) substantially immediately separating the whole plasma from said blood obtained in step (a); and (c) contacting said whole plasma resulting from step (b) with thrombin in a physiologically acceptable solution at a rate and in a volume at the site of treatment to provide fibrin coagulation at said site.

Applicators

15 Application of fibrin glue requires mixing the two components under controlled conditions because gelation can be very rapid. The earliest applicator patents are assigned to Immuno AG. The principal claims revolve around different syringe diameters to limit dilution of the fibrinogen component by using
20 a smaller volume of more highly concentrated thrombin²⁸ and the use of medical gas to keep the syringe applicator/mixer clear of gel²⁹. Galanakis described an application in which the two

-12-

liquids do not mix internally but are applied side-by-side from tubes that run in parallel all the way to the point of application.²⁹ Very similar claims were made previously by Micromedics³⁰. Corus Medical has an aerosol device driven by pressurized gas³¹. The Cryolife approach is to prevent gelation in a single-component fibrin glue by pH inhibition of thrombin³². Gordon Epstein has patented the use of a third suction tip to dry the surface before application³³.

Plasma Separators

10 Haemonetics' "bowl" technology allows separation of plasma continuously by a method which differs from the methods of the present invention disclosed below³⁴. Several patents address isolation of platelets from blood using centrifuge sedimentation methods³⁵ or red cell barriers³⁶, but none claims an approach
15 similar to the ones described herein including sintered (porous) plastic or in which the cells pass through a barrier, leaving platelet-rich plasma behind.

Dextrans

Pharmacia has basic patents on dextranomers³⁶ and on their
20 use in wound healing.³⁷ Interestingly, concentration of proteins (including fibrinogen) in the vicinity of the wound by differential absorption of water and electrolytes is identified

-13-

as a benefit of the technology, but the claims are limited to methods in which the dextranomer beads are applied to the wound.

Other Patents of Interest

Fibrin sealants have been claimed as a component in a composition to repair cartilage³⁸, to help in eye surgery³⁹ and with heat-melted collagen for "tissue welding"⁴⁰. Another approach has been to use fibrinogen/thrombin powder to coat a biodegradable fiber to stop puncture bleeding⁴¹, or in a collagen sponge or mat with powdered fibrin sealant⁴². Addition of collagenase to fibrin sealant to improve nerve healing has been patented by Wehling⁴³. Pharmacia has patented the use of hyaluronic acid to prevent adhesions⁴⁴.

Summary of Current Use of Fibrin Sealants in the United States

Although wounds heal naturally, medicine seeks to improve and speed the wound healing process. The body's blood clotting and tissue repair mechanisms are complex cascades progressing from blood proteins (e.g. fibrinogen) to specialized repair cells (fibroblasts). Sutures, surgical staples or surgical adhesives hold the damaged tissues together so that healing can progress. Surgical "fibrin sealants," sometimes called "fibrin glues," made up of human fibrinogen activated by bovine thrombin are used extensively in Europe. Such fibrin sealants have been shown to

-14-

be superior to synthetic adhesives or traditional surgery in many situations. In addition they reduce the need for blood transfusions. Academic surgical opinion on fibrin sealants is very favorable (see the reviews cited above).

5 In current practice, fibrin sealant is made by isolating a concentrate of human fibrinogen, fibronectin and factor XIII, usually by cryoprecipitation, and combining it immediately before use with bovine thrombin. The thrombin converts the fibrinogen to fibrin which then gels rapidly to form a transient hemostatic
10 plug. The most common method of application of fibrin sealant is by mixing of concentrated fibrinogen from pooled human blood with bovine thrombin and calcium immediately before use.

Fibrin sealant is not available in the U.S. commercially, but is in Europe. The FDA is reluctant to approve these products
15 in the United States because of the potential for virus transmission (e.g., HIV, hepatitis) from pooled human blood and the potential for immunological reactions. These FDA concerns are so serious that the FDA has not approved any fibrin sealant product, despite strong interest from surgeons and very favorable
20 comparative studies in the literature.

1. Hennis, H. L., W. C. Stewart, and E. K. Jeter. "Infectious disease risks of fibrin glue [letter]." Ophthalmic Surg 23 (9 1992): 640.

-15-

2. Berguer, R., R. L. Staerckel, E. E. Moore, F. A. Moore, W. B. Galloway, and M. B. Mockus. "Warning: fatal reaction to the use of fibrin glue in deep hepatic wounds. Case reports." J Trauma 31 (3 1991): 408-11.
- 5 Berruyer, M., J. Amiral, P. Ffrench, J. Belleville, O. Bastien, J. Clerc, A. Kassir, S. Estanove, and M. Dechavanne. "Immunization by bovine thrombin used with fibrin glue during cardiovascular operations. Development of thrombin and factor V inhibitors." J Thorac Cardiovasc Surg 105 (5 1993): 892-7.
- 10 3. Sanal, M. "Does fibrin glue cause foreign body reactions? [letter]." Eur J Pediatr Surg 3 (3 1993): 190.
Sanal, M., H. Dogruyol, A. Gurpinar, and O. Yerci. "Does fibrin glue cause foreign body reactions?" Eur J Pediatr Surg 2 (5 1992): 285-6.
- 15 4. Thompson, D. F., Letassy, N. A., and Thompson, G. D., Fibrin glue: a review of its preparation, efficacy, and adverse effects as a topical hemostat. Drug Intell Clin Pharm, 1988. 22(12): p. 946-52.
- 20 5. Siedentop, K. H., D. M. Harris, and B. Sanchez. "Autologous fibrin tissue adhesive." Laryngoscope 95 (9 Pt 1 1985): 1074-6.
6. Epstein, G. H., R. A. Weisman, S. Zwillenberg, and A. D. Schreiber. "A new autologous fibrinogen-based adhesive for otologic surgery." Ann Otol Rhinol Laryngol 95 (1 Pt 1 1986): 40-5.
- 25 7. Weis-Fogh, U. S. "Fibrinogen prepared from small blood samples for autologous use in a tissue adhesive system." Eur Surg Res 20 (5-6 1988): 381-9.
8. Hartman, A. R., D. K. Galanakis, M. P. Honig, F. C. Seifert, and C. E. Anagnostopoulos. "Autologous whole plasma fibrin gel. Intraoperative procurement." Arch Surg 127 (3 1992): 357-9.
- 30 9. Kjaergard, H. K., Fogh Us Weis, and J. J. Thiis. "Preparation of autologous fibrin glue from pericardial blood." Ann Thorac Surg 55 (2 1993): 543-4.
- 35 Kjaergard, H. K., U. S. Weis-Fogh, H. Sørensen, J. Thiis, and I. Rygg. "A simple method of preparation of autologous fibrin glue by means of ethanol." Surg Gynecol Obstet 175 (1 1992): 72-3.

-16-

10. Casali, B., F. Rodeghiero, A. Tosetto, B. Palmieri, R. Immovilli, C. Ghedini, and P. Rivasi. "Fibrin glue from single-donation autologous plasmapheresis." *Transfusion* 32 (7 1992): 641-3.

5 Moretz, W, Jr., J Shea Jr., J. R. Emmett, and J Shea. "A simple autologous fibrinogen glue for otologic surgery." Otolaryngol Head Neck Surg 95 (1 1986): 122-4.

10 11. Matras, Helene, H. P. Dinges, H. Lassmann, and B. Mamoli. "Zur nahtlosen interfazikularen Nerventransplantation im Tierexperiment." *Wein Med Wochschr* 122 (37 1972): 517-523.

First clinical results: Kuderma, H. and Helene Matras. "Die klinische Anwendung der Klebung von Nervenastomosen mit Gerinnungssubstanzen bei der Rekonstruktion verletzter peripherer Nerven." *Wein Klin Wochenschr* 87 (15 1975): 495-501.

15 12. Schwarz, O., Linnau, Y., Loblich, F., and Seelich, T., *Tissue adhesive; freeze dried wound healing agent containing Fibrinogen, albumin, factor XIII, globulin, and plasminogen blocking Agent*. US Patent 4,414,976 (1983); Schwarz, O., Linnau, Y., Loblich, F., and Seelich, T., *Tissue adhesive; factor viii, proteins, fibrinogen, globulin, albumin and plasmin inhibitor*. US
20 Patent 4,377,572 (1982); Schwarz, O., Linnau, Y., Loblich, F., and Seelich, T., *Tissue adhesive; fibrinogen, factor XIII, albumin, plasmin inhibitor*. US Patent 4,362,567 (1982); Schwarz, O., Linnau, Y., Loblich, F., and Seelich, T., *Tissue adhesive; blood proteins; surgery*. US Patent 4,298,598 (1981); assigned to
25 Immuno AG.

13. Martinowitz, U. and Bal, F., *Improved tissue glue prepared by using cryoprecipitate*. EP (application) Patent 534,178 (1993) Assigned to Octapharma AG.

30 14. Stroetmann, M., *Enriched plasma derivative for advancement of wound closure and healing*. US Patent 4,427,650 (1984); Stroetmann, M., *Fibrinogen-containing dry preparation, manufacture and use thereof*. US Patent 4,442,655 (1984); Stroetmann, M., *Enriched plasma derivative for enhancement of wound closure and coverage*. US Patent 4,427,651 (1984); assigned
35 to Serapharm Michael Stroetmann DE.

15. Fuhge, P., Heimberger, N., Stohr, H.-A., and Burk, W., *Readily Dissolvable Lyophilized Fibrinogen Formulation*. US Patent 4,650,678 (1987) Assigned to Behringwerke Aktiengesellschaft.

-17-

16. Burnouf-Radosevich, M. and Burnouf, T., *Concentrate of thrombin coagulable proteins, the method of obtaining same and therapeutical use thereof*. US Patent 5,260,420 (1993) Assigned to Centre Regional de Transfusion Sanguine de Lille.

5 17. Epstein, G. H., *Method and apparatus for preparing fibrinogen adhesive from whole blood*. US Patent 5,226,877 (1993).

18. Morse, B. S., Carpenter, J. F., Turner, A. D., and Cryolife, I., *Preparation of fibrinogen/factor XIII precipitate*. US Patent 5,030,215 (1991) Assigned to Cryolife. Claim 1: A system for
10 collecting a blood coagulation factor comprising a first container means for receiving whole blood, said first container means having an upper end, a lower end, at least one inlet port, add at least one outlet port; first conduit means for conveying
15 whole blood to said first container means, said first conduit means having an end thereof coupled to a said inlet port of said first container means; second container means for receiving plasma which has been separated from red blood cells in said first container means, said second container means having an
20 upper end, the container means having a first, relatively wide diameter portion adjacent to said upper end thereof, a second relatively narrow portion defined below said first portion for receiving a blood coagulation factor precipitate from the plasma within said second container means and a third relatively wide
25 portion defined below said second, relatively narrow portion so that said second, relatively narrow portion defines a relatively narrow passage for precipitate blood coagulation factor from said first portion to said third portion; and second conduit means having a first end thereof coupled to a said outlet port of said
30 first container means and a second end thereof coupled to a said inlet port of said second container means for conveying plasma from said first container means to said second container means.

19. Sierra, D. H., Brown, D. M., and Luck, E. E., *Surgical adhesive material*. US Patent 5,290,552 (1994) Assigned to Matrix Pharm. Inc/Project Hear.

35 20. Wadstroem, J., *Tissue treatment composition comprising fibrin or fibrinogen and biodegradable and biocompatible polymer*. WO Patent 92/22,312 (1992) .

21. Iwatsuki, M. and Hayashi, T., *Silk-fibrin and human-fibrinogen adhesive composition*. US Patent 4,818,291 (1989)
40 Assigned to Ajinomoto Co Inc.

-18-

22. Tse, D. C., Alpern, M., Enomoto, S. T., Garanchon, C. M., Liu, S. L., Mankarious, S. S., and Thomas, W. R., *Topical fibrinogen complex*. WO Application Patent 05067 (1993) Assigned to Baxter Int Inc.

5 23. Edwardson, P. A. D., Fairbrother, J. E., Gardner, R. S., Hollingsbee, D. A., and Cederholm-Williams, S. A., *Fibrin sealant compositions and method for utilizing same*. EP (Application) Patent 592,242 (1993) Assigned to Squibb.

10 24. Rose, E. and Dresdale, A., *Method of preparing a cryoprecipitated suspension and use thereof*. US Patent 4,928,603 (1990) Assigned to Columbia University. Claim 1: A method of preparing a cryoprecipitated suspension containing fibrinogen and Factor XIII useful as a precursor in the preparation of a fibrin glue which consists essentially of: (a) freezing fresh frozen
15 plasma from a single donor which has been screened for blood transmitted diseases at about -80° C. for at least 6 hours, (b) raising the temperature of the frozen plasma so as to form a supernatant and a cryoprecipitated suspension containing
20 fibrinogen and Factor XIII, and (c) recovering the cryoprecipitated suspension.

Rose, E. and Dresdale, A., *Fibrin adhesive prepared as a concentrate from single donor fresh frozen plasma*. US Patent 4,627,879 (1986) Assigned to Columbia University. Summary:
25 Prodn. of a fibrin glue comprises preparing a cryoprecipitated suspension contg. fibrinogen and Factor XIII by freezing fresh frozen plasma from a single donor which has been screened for blood transmitted diseases at -80 deg. C for at least 6 hrs., raising the temp. to form a supernatant and cryoprecipitated
30 suspension contg. fibrinogen and Factor XIII, and recovering the suspension. A defined vol. of the suspension is applied to the desired site and a compsn. contg. thrombin is applied to cause the fibrinogen in the suspension to be converted to the fibrinogen glue, which then solidifies as a gel.

35 25. Weis-Fogh, U., *A Method and an Apparatus for preparing tissue repair promoting substances*. WO (Application) Patent 88/02259 (1988).

40 26. Alterbaum, R., *Method and apparatus for use in preparation of fibrinogen from a patient's blood*. US Patent 4,714,457 (1987). Claim 1: A method for use in the autologous preparation of fibrin glue wherein a patient's blood is separated in a centrifuge having cylindrical cups pivotally mounted to a rotor to obtain plasma and wherein the plasma is separated in the centrifuge to produce concentrated fibrinogen, comprising the steps of: (a)

-19-

transferring the blood or plasma into a substantially flat packet; (b) fixing the packet containing the blood or plasma in a recess of a substantially cylindrical insert fixture assembly; (c) inserting said cylindrical-shaped insert fixture assembly in a cup of the centrifuge having a complementary cylindrical shape so that the insert fixture assembly is held snugly within said centrifuge cup; (d) centrifuging the blood or plasma contained within said packet fixed in said insert fixture assembly held in said centrifuge cup to separate the blood or plasma into components; and (e) forming fibrin glue from one of said separated components.

27. Galanakis, D. K., *Method of preparing autologous plasma fibrin and application apparatus therefore*. US Patent 5,185,001 (1993) Assigned to Univ New York State Res Found.

28. Eibl, J., Habison, G., Redl, H., and Seelich, T., *Arrangement for applying a tissue adhesive*. US Patent 4,735,616 (1988) Assigned to Immuno AG.

29. Redl, H. and Habison, G., *Apparatus for Applying a tissue adhesive*. US Patent 4,631,055 (1986) Assigned to Immuno AG.

30. Miller, C. H., Altshuler, J. H., and Arenberg, I. K., *Fibrin glue delivery system*. US Patent 4,874,368 (1989) Assigned to Micromedics.

31. Avoy, D. R., *Fibrinogen dispensing kit*. US Patent 4,902,281 (1990) Assigned to Corus Med Corp.

32. Morse, B. S., McNally, R. T., and Turner, A. D., *Fibrin sealant delivery method*. US Patent 5,219,328 (1993) Assigned to Cryolife.

33. Headley, T. D., *Plasmapheresis centrifuge bowl; disposable*. US Patent 4,983,158 (1991) Assigned to Haemonetics Corp; Pages, E., *Disposable centrifuge bowl for blood processing*. US Patent 4,943,273 (1990); Latham, A., Jr., *Apparatus for separating blood into components thereof*. US Patent 4,303,193 (1981); Latham, A., Jr., *Process for pheresis procedure and disposable plasma*. US Patent 4,204,537 (1980); Latham, A., Jr., *Process for pheresis procedure and disposable pheresis bowl therefor*. US Patent 4,059,108 (1977); assigned to Haemonetics.

34. Pall, D. B. and Gsell, T. C., *Method for obtaining platelets*. US Patent 5,258,126 (1993) Assigned to Pall Corp; Pall, D. B. and Gsell, T. C., *Blood collection and processing system*. US Patent 5,100,564 (1992) Assigned to Pall Corp.

-20-

35. Pall, D. B., Gsell, T. C., Matkovich, V. I., and Bormann, T., System and method for processing biological fluid. US Patent 5,217,627 (1993) Assigned to Pall Corp; Pall, D. B., Gsell, T. C., and Muellers, B. T., Method for processing blood for human transfusion. US Patent 5,152,905 (1992) Assigned to Pall Corp; Eldegheidy, M. M., Automatic liquid component separator. US Patent 4,639,316 (1987) Assigned to Becton Dickinson & Co.
36. Process for the Manufacture of Hydrophilic High Molecular Weight Substances from Dextran Substances. GB Patent 854,715 (1965); Gelotte, E. B. and Soderquist, B. G. F., [Hydroxy compound copolymers dextran-epichlorhydrin] Verfahren zur Herstellung von Substitutionsprodukten von Mischpolymerisaten. DD Patent 56,103 (1965); Beil, W., Hoepfner, A., Wolff, H. J., and Beil, H., [Verfahren zur Herstellung von hochmolekularen hydrophilen Vernetzungsprodukten von Polysacchariden oder deren Derivaten oder von Polyvinylalkohol in Form von Gelkornern] High mol. wt. hydrophilic copolymer of a hydroxy group-contng., non-ionic polymer is obtd. in the form of gel grains by reacting the polymer, in the presence. DE Patent 1,443,359 (1962); assigned to Pharmacia.
37. Rothman, U. S. and Jacobsson, S. A., Method for cleansing fluid discharging skin surfaces, wounds and mucous membranes and means for carrying out the method; dry particles of water-insoluble swellable polymer. US Patent 4,537,767 (1985) Assigned to Pharmacia AB; Rothman, U. S. and Jacobsson, S. A., Method for cleansing fluid discharging skin surfaces, wounds and mucous membranes and means for carrying out the method. US Patent 4,225,580 (1980) Assigned to Pharmacia AB.
38. Hunziker, E. B., Method and compositions for the treatment and repair of defects or lesions in cartilage. US Patent 5,206,023 (1993).
39. Sarfarazi, F., Sarfarazi method of closing a corneal incision. US Patent 5,190,057 (1993); O'Donnell, F., E., Jr., Mammer, E., and Nalbandian, R. M., Intraocular lens implant and method of locating and adhering within the posterior chamber. US Patent 5,002,571 (1991).
40. Sawyer, P. N., Collagen welding rod material for use in tissue welding. US Patent 5,156,613 (1992) Assigned to Interface Biomedical Laboratories.
41. Sakamoto, I., Unigame, T., and Takagi, K., Hemostatic agent. US Patent 4,655,211 (1987) Assigned to Unitika KK.

-21-

42. Zimmermann, E. and Schiele, U., Agent for sealing and healing wounds. US Patent 4,453,939 (1984) Assigned to Hormon-Chemie Munch; Stemberger, A., Gewebeverklebbarre kollagene Wundauflage. EP Patent 102,773 (1983) Assigned to Dr. Ruhland Nachf. GmbH.
- 5
43. Wehling, P., Method of enhancing the regeneration of injured nerves and adhesive pharmaceutical formulation therefor. US Patent 5,279,825 (1994) Assigned to Advanced Biofactures; Wehling, P., Method of enhancing the regeneration of injured nerves and adhesive pharmaceutical formulation therefor; supplying collagenase to the zone of nerve injury during regeneration. US Patent 5,173,295 (1992) Assigned to Advanced Biofactures.
- 10
44. Lindblad, G. and Buckley, P., Composition and method for prevention of adhesions between body tissues. US Patent 5,190,759 (1993) Assigned to Kabi Pharmacia AB.
- 15

-22-

SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide a novel method and apparatus for rapidly preparing a plasma concentrate from blood containing fibrinogen, prothrombin
5 (the inactive precursor of thrombin) and other blood proteins, and platelets (thrombocytes) (citrate, heparin or other anticoagulant may be present in an amount sufficient to prevent initiation of clotting).

10 A further object of the present invention is to provide a novel method and apparatus for rapidly preparing a plasma concentrate from a patient's own blood (autologous tissue sealant).

15 A further object of the present invention is to provide a novel method and apparatus for rapidly preparing a concentrate from a patient's own blood while in the operating room (stat autologous tissue sealant).

A further object of the present invention is to provide a composition with physical, chemical and biological properties superior to plasma protein precipitate derived tissue sealants.

20

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following

-23-

detailed description when considered in connection with the accompanying drawings, wherein:

Fig. 1 shows an embodiment of a disposable cartridge in accordance with the present invention, employing plasma separation by open cell hydrophobic foam and plasma concentration
5 by open cell foam with beads held thereto by electrostatic force;

Fig. 2 shows an embodiment of a disposable cartridge in accordance with the present invention, employing plasma separation by sintered plastic barrier and plasma concentration
10 by beads distributed in a trough;

Fig. 3 shows an embodiment of a disposable cartridge in accordance with the present invention, employing plasma separation by annular chamber and plasma concentration by open
cell foam with beads held thereto by electrostatic force;

15 Fig. 4 shows an embodiment of a disposable cartridge in accordance with the present invention, employing plasma separation by felt mat and plasma concentration by beads impregnated into plastic discs;

20 Fig. 5 depicts a single enlarged concentrator bead in a solution of low molecular weight and macromolecule components (e.g., plasma) in accordance with the present invention; and

Fig. 6 shows an embodiment of a disposable cartridge in accordance with the present invention after centrifugation and during syringe withdrawal of the plasma concentrate;

-24-

Fig. 7 shows an embodiment of the applicator in accordance with the present invention employing coaxial needles to allow mixing of the two components;

Fig. 8 shows an embodiment of the applicator in accordance with the present invention employing an activator cartridge to
5 allow application of single component fibrin sealant; and

Fig. 9 graphically shows the relationship between the concentration of proteins and their molecular weight, for a protein solution or suspension concentrated 3X with beads having
10 a molecular weight cutoff of 10 kDa in accordance with the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

An inexpensive device with a disposable cartridge for preparation of tissue sealant is disclosed. The device is
15 particularly applicable to stat preparation of autologous tissue sealant. The disposable cartridge may fit in the palm of the hand and is hermetically sealed to eliminate possible exposure to patient blood and ensure sterility. Methods of sealing tissue in which the tissue sealant is applied immediately after mixing
20 platelet-rich plasma concentrate (from the device) with a solution of calcium and thrombin or in which the tissue sealant is applied immediately after physical or immobilized enzyme activation are disclosed.

-25-

Preparation in the operating room of 5cc sealant from 50cc patient blood requires less than 15 minutes and only one simple operator step. There is no risk of tracking error because processing can be done in the operating room. Additional
5 chemicals used in the plasma sealant and preparation thereof may be limited to anticoagulant (for example, citrate) and calcium salts (e.g., chloride).

Adhesive and tensile strengths are comparable to pooled blood fibrin sealants that are available in Europe commercially.
10 Antifibrinolytic agents (such as aprotinin) are not necessary because the tissue sealant contains high concentrations of natural inhibitors of fibrinolysis from the patient's blood. The tissue sealant also contains patient platelets and additional factors not present in available fibrin sealants that promote
15 wound healing.

The present (autologous) tissue sealant gels rapidly as it is mixed with calcium (and optionally bovine or human thrombin) while being applied to the wound. This sealant contains only FDA-approved or approvable components. In the present invention,
20 a "tissue sealant" contains the components found in blood that contribute to clot formation, tissue adhesion and wound healing (preferably all such components in blood), and thus, is distinguished from prior "fibrin sealants" which typically contain only cryoprecipitated proteins and no platelets.

-26-

The present invention concerns a method of making platelet-rich plasma (PRP) concentrate, which can then be combined with calcium/thrombin to make tissue sealant. The resulting gel is a composition that has never before been described in the scientific or patent literature.

Referring now to the drawings, wherein like reference numerals designate identical or corresponding parts throughout the several views, and more particularly to Figure 1 thereof, in a preferred embodiment of the present invention, citrated plasma is processed in two stages in a disposable centrifuge cartridge. In a first stage, platelet-rich plasma is separated from cells when centrifugal force causes red and white cells to lodge irreversibly in a first separator (e.g., a hydrophobic open cell foam). In a second stage, the platelet-rich plasma is concentrated by contact with a concentrator (e.g., dextranomer beads) that absorbs water, electrolytes and small proteins, leaving a platelet-rich plasma concentrate. Thus, the present method of making platelet-rich plasma comprises the steps of:

- (a) separating plasma and platelets from whole blood;
- (b) contacting the plasma and platelets with a concentrator to provide the concentrated platelet-rich plasma; and
- (c) separating the concentrated platelet-rich plasma from the concentrator.

-27-

Preferably, the cartridge is disposable and sealed (e.g., see housing 1 in each of Figs. 1 through 4), making contamination impossible. Where the method is performed in a sealed cartridge, the separating step (a) above may be conducted, for example, in a first chamber of the cartridge which is in fluid communication with the blood fill port (inlet). The plasma and platelets may then be contacted with a concentrator (e.g., in a second chamber of the cartridge in fluid communication with the first chamber) sufficiently to concentrate the plasma. An advantage of the sealed, disposable cartridge is that it can be used in the operating room, thus eliminating the possibility of tracking errors and contamination.

In a further embodiment, the method is one for making autologous concentrated platelet-rich plasma, in which the method above for making concentrated platelet-rich plasma further comprises, prior to the separating step (a), collecting blood from a patient and adding an effective amount of citrate to inhibit coagulation (in accordance with standard methods), and injecting a standard quantity of blood (e.g., 50cc) into the blood fill port (inlet) 5 of a cartridge 1.

Plasma is separated from blood by a combination of conventional centrifugation technology and inventions for ease of separation of the packed cells from the plasma. Centrifuges which are capable of spinning at variable speeds up to 3,000-

-28-

10,000 rpm in either direction and which are capable of being controlled by a microprocessor (i.e., for spinning at predetermined rates of rotation for predetermined lengths of time) are known and are easily adapted, if necessary, for use with the present apparatus for separating and concentrating plasma. Following injection of the whole blood, the operator starts the microprocessor-controlled centrifuge by push-button. All remaining steps until removal of the concentrate may be automatic.

Although cells are conventionally separated from plasma by their property of packing into a cell mass during centrifugation (then typically aspirated), the cartridges are designed to effect rapid separation of the plasma into the next, concentrator chamber. In one embodiment (Fig. 1), red and white cells remain trapped in a hydrophobic open cell foam 14. In another embodiment (Fig. 4) red and white cells remain trapped in a felt annulus 7. In a third embodiment (Fig. 2) the cells pass through sinuous pathways in a sintered annulus 8 and remain trapped outside. In a fourth embodiment (Fig. 3) red and white cells migrate through a small gap 16 and remain trapped in an outer annulus by the force of gravity when the centrifuge stops.

Optionally, the plasma may be contacted with dextranomer beads that have been bonded by electrostatic force with an open, hydrophobic cellular foam 15 (in accordance with the apparatus of

-29-

Figs. 1 and 3). When wetted the beads lose their electrostatic attraction and begin to swell. Alternatively, the plasma may be contacted with free dextranomer beads 2, e.g., in accordance with the apparatus of Fig 2. The centrifuge may rotate briefly
5 clockwise then counterclockwise a few times at a speed sufficient to evenly distribute the free dextranomer beads 2 outside the concentrator cup lip 3.

The centrifuge may then spin at from 1,000 to 10,000 rpm, preferably 3,000 rpm, for one to ten minutes to separate
10 platelet-rich plasma from cells. First, the whole blood passes through a pore 4 in the blood inlet 6 into the separation chamber. Cells may become trapped in a matrix 14 (as shown in Fig. 1), enmeshed in a matrix 7 (as shown in Fig. 4), or pass through sintered hydrophobic plastic or other porous material 8
15 (as shown in Fig. 2), leaving platelet-rich plasma in the chamber.

The centrifuge may then be stopped for 15-90 seconds. Under the force of gravity, the platelet-rich plasma passes through an annulus (or through a hydrophilic filter 9) into the second
20 chamber 10 (the lower chamber in Figs. 1 through 4). This chamber may also be identified as a first concentrator chamber.

In the first concentrator chamber, platelet-rich plasma comes into contact with the concentrator (e.g., dextranomer or polyacrylamide), either bonded by electrostatic force with an

-30-

open, hydrophobic cellular foam 15 (Figs. 1 and 3) or free beads 2 (Fig. 2) or beads embossed onto disc(s) 11 (Fig. 4).

Dextranomer and polyacrylamide concentrators are commercially available (as SEPHADEX from Pharmacia and as BIO-GEL P from Bio-
5 Rad Laboratories, respectively). Alternatively, instead of dextranomer or polyacrylamide absorbent beads, other concentrators, such as SEPHADEX moisture or water absorbants (available from Pharmacia), other polyacrylamide absorbants, silica gel, zeolites, dextramines, alginate gel, starch, cross-
10 linked agarose, etc., in the form of beads or discs are also acceptable concentrators.

In essence, any material that absorbs water (i.e., which is sufficiently dry to permit water absorption) and which does not denature the proteins essential to blood clotting (e.g.,
15 fibrinogen and factor XIII) can be used as a concentrator, as can appropriately-selected membranes, which may also employ an osmotic gradient. Further, where the centrifuge has autoclave-like capabilities (i.e., the ability to rotate its contents at elevated temperatures and/or atmospheric pressures other than 1
20 atm), mechanical concentrators, such as an elevated temperature (e.g., a temperature of 30-80°C, preferably 35-60°C) or a reduced pressure/vacuum (e.g., 0.1-500 Torr, preferably 1-200 Torr) may serve as a concentrator. However, beads employed as described both above and hereunder are the preferred concentrator.

-31-

To maximize results, initial slow rotation of the centrifuge aids mixing (and thus contact) of the concentrator and the plasma. "Slow rotation" refers to a centrifuge speed of from 20 rpm to 500 rpm, preferably from 100 rpm to 300 rpm.

5 The porosity and hydrophobicity of the porous wall 7, 8 or 14 are such that the low centrifugal force generated is sufficient to drive plasma up the wall but is not sufficient to overcome surface tension, and the plasma remains with the compartment at the inner surface.

10 The beads 2 swell with water, ions, and low molecular weight proteins 51 (see Fig. 5). The size of the pores 52 in the concentrator bead 2 is chosen so that the higher molecular weight proteins 53 become concentrated in the space around the beads. Any commercially available bead concentrators for concentrating
15 proteins having a molecular weight above a certain preselected molecular weight cutoff value may be used.

As those of ordinary skill in protein chromatography understand, a "molecular weight cutoff" refers to pores in a matrix (e.g., SEPHADEX) which are distributed about a mean size.
20 Moieties (e.g., proteins) more than, for example, 25-50% smaller than mean size diffuse into the bead rapidly, and moieties more than, for example, 25-50% larger than mean size do not diffuse into the bead. Proteins having a size within, for example, 25-50% of the mean size diffuse into the bead slowly (depending on

-32-

the particular bead selected). Thus, a bead having a molecular weight cutoff of 10 kDa concentrates proteins according to molecular weight as shown graphically in Figure 9.

Consequently, in the present application, "high molecular weight compounds" refer to those compounds having a molecular weight greater than the preselected molecular weight cutoff value of the concentrator. Examples of high molecular weight proteins in plasma may include fibrinogen and factor XIII, depending on the selected molecular weight cutoff. Preferred concentrator materials include those beads or materials having a molecular weight cutoff value of 100 kDa, preferably 30 kDa and more preferably 5 kDa.

It is important to note that the platelet-rich plasma concentrate contains all components of the original plasma, in either the original concentration (ions and compounds with molecular weight less than the concentrator cutoff) or at a greater concentration than naturally-occurring plasma (e.g., platelets and compounds with molecular weight more than the concentrator cutoff). The protein concentrate contains the same ion concentrations as the unconcentrated plasma.

The quantity of beads is chosen so that the platelet rich plasma is concentrated by a desired amount, e.g., 3X (the approximate level of concentration achieved by a predetermined volume of fluid surrounding close-packed spheres in the same

-33-

volume). When using DEBRISAN (trademark, Johnson and Johnson), approximately 2.6 gm is required per 10cc plasma to achieve 3-fold concentration.

After absorption of water and low molecular weight solutes
5 into the concentrator, the rate of rotation is increased (e.g.,
to 1,000-10,000 rpm, preferably to about 3,000 rpm), forcing the
concentrated platelet-rich plasma out through a filter 12 into an
outer compartment. The centrifuge may then be stopped, and under
the force of gravity, concentrated platelet-rich plasma may be
10 collected in a bottom well 13. The concentrated platelet-rich
plasma may be left for extended periods (e.g., hours) without
spontaneous gelation or other deleterious effect.

Concentrated platelet-rich plasma, ready for use, may then
be removed into a syringe 16 as in Fig. 6.

15 Thus, the present invention also concerns a method of
sealing tissue, comprising the steps of contacting the present
concentrated plasma with an activator (either a thrombin/calcium
solution or disrupted platelets as described below) to provide an
activated plasma concentrate, and applying the activated plasma
20 concentrate to tissue in need of sealing.

In one embodiment of the method of sealing tissue, a
separately-prepared solution containing amounts of thrombin and
calcium effective to coagulate the concentrated plasma may be
combined with the concentrated plasma (in accordance with methods

-34-

described in the literature for cryoprecipitated fibrinogen). Once combined, the solution and concentrated plasma rapidly gel to form a tissue sealant. An embodiment with features superior to any previously described is shown in Fig. 7. In the barrel of the syringe a cylindrical chamber 31 containing thrombin and calcium solution is surrounded by an annular chamber 32 containing concentrated platelet-rich plasma. Plunger 30 causes the solutions to exit the apparatus through a coaxial needle, 33 (thrombin) and 35 (concentrated plasma), and mix at the tip 36. An optional sleeve 34 ensures proper mixing, and may be retracted to expose premature gel for easy removal.

In a further optional embodiment, a needle tip designed to activate fibrinogen may be used to apply a single-component concentrate (i.e., the concentrated plasma) without the use of added thrombin. In this embodiment, calcium is added to the concentrate immediately prior to or during its application to tissue in need of sealing. Fig. 8 shows a syringe 21 and applicator needle tip 23 separated by activator cartridge 22 through which platelet-rich plasma passes for activation by immobilized proteolytic enzyme, e.g., bovine thrombin.

In a further optional embodiment, a needle tip designed to disrupt platelets may be used to apply a single-component concentrate (i.e., the concentrated plasma) without the use of thrombin. In this embodiment, calcium is added to the

-35-

concentrate immediately prior to or during its application to tissue in need of sealing. Fig. 8 shows a syringe 21 and applicator needle tip 23 separated by activator cartridge 22 through which platelet-rich plasma passes for activation by, e.g., nylon wool.

The present invention also concerns an apparatus for concentrating platelet-rich plasma, comprising:

an inlet,

a first chamber in fluid communication with the inlet, containing a first separator for separating plasma and platelets from whole blood, thus forming platelet-rich plasma,

a second chamber in fluid communication with the first chamber, containing a concentrator for concentrating the platelet-rich plasma and a second separator for separating the concentrated platelet-rich plasma from the concentrator, and

an outlet for withdrawing the concentrated platelet-rich plasma.

In a further embodiment, the first separator comprises an open cell hydrophobic foam which traps red blood cells and white blood cells therein under surface tension force.

In a further embodiment, the first separator comprises a porous plastic wall which allows passage of red blood cells and white blood cells therethrough under a centrifugal force, but

-36-

does not permit passage of red blood cells and white blood cells in the absence of the centrifugal force.

In a further embodiment, the first separator comprises an annulus at the top of the first chamber that allows red blood
5 cells and white blood cells to pass into an outer annulus and be separated from plasma, maintained by gravity when the centrifuge stops.

In a further embodiment of the apparatus, the concentrator may be embedded onto a paper disc, or onto a plurality of discs
10 (of any commercially available material), or may stick electrostatically or by other forces to an open cell matrix.

Many designs of a preferred embodiment of the present cartridge are envisioned (for example, Figs 1 through 4). The designs of the first and second chambers are largely independent,
15 and may be combined into a single cartridge at will. All four designs shown have at least one separation chamber in fluid connection with at least one concentration chamber.

The most preferred embodiment, Fig. 1, uses open cell hydrophobic foam 14 for separation of plasma from cells and
20 effects plasma concentration by beads held by electrostatic force in an open cell foam 15. Another preferred embodiment, shown in Fig. 2, uses plasma separation by sintered plastic barrier 8 and plasma concentration by beads distributed in a trough 2. Another preferred embodiment, shown in Fig. 3, uses plasma separation by

-37-

annular chamber 16 and plasma concentration by beads held by electrostatic force within open cell foam 15. Another preferred embodiment, shown in Fig. 4, uses plasma separation by felt mat 7 and plasma concentration by beads impregnated into plastic discs 12.

Each separator can be used with each concentrator. The four separators may be combined with the three concentrators to give twelve possible arrangements for the disposable cartridge.

A. Open Cell Hydrophobic Foam Plasma Separator and Dextranomer Bead in Open Cell Hydrophobic Foam Concentrator

In Figure 1, the ready cartridge is shown in the right half and the path of the liquid is shown by the dashed arrows on the left half. The entire chamber is radially symmetric except the septum 5 for the whole blood inlet. The receiving cup 6 holds blood until centrifugal force causes blood to pass through the filter 4. In the separation chamber, centrifugal force causes cells to pass into the open cell hydrophobic foam 14 and accumulate therein; continuing centrifugal force causes cells to migrate into the annulus of open cell foam and platelet rich plasma to remain within the separation chamber. The open cell hydrophobic foam 14 is a honeycomb-like hydrophobic material which allows fluids and small particles to flow freely (e.g., open cell polyurethane foam). Like a wetted sponge, the foam

-38-

holds liquid against a certain head of pressure due to surface tension forces. Thus, blood cells or other suspended particulates remain entrapped within the foam when the centrifuge stops and separated platelet-rich plasma drains from the surface under the force of gravity. Foam can be either rigid or flexible and can be formed into the appropriate annular shape for the device by molding or die-cutting. The parts are sized so that the packed cell (e.g., erythrocyte and leukocyte) layer is fully contained within the outer open cell foam chamber, which retains the cells when the centrifuge stops.

Concentration comes about when platelet-rich plasma contacts concentrator (e.g., dextranomer) beads 2 in the concentration chamber 10. These beads may be a molecular sieve used in chromatography (SEPHADEX, available from Pharmacia; BIO-GEL P, available from Bio-Rad Laboratories) and debriding (DEBRISAN, available from J&J), or may comprise silica gel, zeolites, dextramines, alginate gel, starch, cross-linked agarose, etc. DEBRISAN appears similar to SEPHADEX G-25, and both are available commercially.

The beads are held by static electric forces onto the surface of open cell hydrophobic foam 15. The open cell hydrophobic foam 15 is a honeycomb-like hydrophobic material. After the platelet-rich plasma has entered the concentration chamber 10, the cartridge 1 is gently rotated to mix platelet-

-39-

rich plasma with concentrator beads 2. As the fluid penetrates the open foam 15, the beads lose their affinity for the foam and begin to swell. Foam 15 may be made from the same material as foam 14 (e.g., open cell polyurethane), but may have a different porosity. The porosity of foams 14 and 15 is selected empirically on the basis of the sizes and/or types of particles to be retained (e.g., cells by foam 14 and concentrator beads by foam 15). In commercially available open cell, three-dimensional polyurethane foams, porosity is known to be correlated to density (which is measured in, e.g., weight per cubic foot).

After a few minutes (e.g., 1-10 min., preferably 3-5 min.), the concentration is complete. Note that concentration by approximately three times corresponds to fluid surrounding close-packed spheres; thus, three times concentration is optimal. Concentration of more than three times is possible, but to achieve best results, a second concentration chamber should be used for further concentration of plasma concentrated in a first chamber. Thus, concentration of nine times is possible for a three-chamber cartridge containing two concentration chambers.

When concentration is complete, the centrifuge increases its rate of rotation and the increased centrifugal forces cause concentrated platelet-rich plasma to pass through the hydrophobic annulus 12. Sintered plastic 12 (e.g., POREX) is comprised of small particles of plastic fused to create a porous structure.

-40-

Fluids and small particulates can flow freely through the tortuous pathways defined by the spaces between the fused plastic particles when the material is wet. If the plastic is not specially formulated or treated, the sintered plastic will be hydrophobic and when in the dry state will afford resistance to influx of water, more greatly as the size of the pathways is reduced. This property of "entry pressure" allows the wetting of the surface of a hydrophobic porous wall without substantial entry of fluid into the material. At greater pressure, i.e., when centrifugal force is increased, this resistive force is overcome and fluid breaks through the barrier to enter and pass through the porous wall.

The centrifuge stops, and concentrated platelet-rich plasma runs to the bottom under the force of gravity and is ready to be removed through filter 13 as in Fig 6.

Finally, note that the concentrator cup 10 is shaped like an inverted funnel to vent the lower chamber to the upper chamber allowing displacement of air from the lower chamber by plasma.

B. Hydrophobic Porous Wall Plasma Separator and Free Dextranomer Bead Concentrator

In Figure 2, the ready cartridge is shown in the right half and the path of the liquid is shown by the dashed arrows on the left half. The entire chamber is radially symmetric except the

-41-

septum 5 for the whole blood inlet. The receiving cup 6 holds blood until centrifugal force causes blood to pass through the filter 4. In the separation chamber, centrifugal force causes cells to pass through the sinuous channels of filter 8 and
5 accumulate in the outer annulus. The filter 8 (e.g., 80 μ POREX) is comprised of small particles of plastic fused to create a porous structure. Fluids and small particulates can flow freely through the tortuous pathways defined by the spaces between the fused plastic particles when the material is wet. If the plastic
10 is not specially formulated or treated, the sintered plastic will be hydrophobic and when in the dry state will afford resistance to influx of water, more greatly as the size of the pathways is reduced. This property of "entry pressure" allows the wetting of the surface of a hydrophobic porous wall without substantial
15 entry of fluid into the material. At greater pressure, i.e., when centrifugal force is increased (e.g., at 1000-10,000 rpm or higher), this resistive force is overcome and fluid breaks through the barrier to enter and pass through the porous wall. This leaves plasma and platelets in the chamber. The parts are
20 sized so that the packed cell (e.g., erythrocyte and leukocyte) layer is fully contained within the filter and outer annulus. When the centrifuge stops and the platelet-rich plasma passes through the hydrophilic funnel filter 9, the force of gravity is

-42-

insufficient to cause cells to pass through the filter 8 and back into the separation chamber.

Hydrophilic funnel filter 9 separates the upper and lower compartments so that the loose beads cannot tumble into the upper compartment during shipping and handling. This material might, for example, be similar to that used for filter 8. In order that entry pressure not be so high as to preclude free drainage of plasma (by gravity) into the lower compartment after separation from blood cells, however, this material would preferably be of effective porosity minimally sufficient to ensure entrapment of beads in the concentrator chamber 10. The material would also most desirably be treated in a manner such as to render it more hydrophilic, (e.g., plasma or corona treated or blended or coated with surfactant) in order to further reduce entry pressure.

Concentration comes about when platelet-rich plasma contacts concentrator beads 2 in the concentration chamber 10. These beads may be a molecular sieve used in chromatography (SEPHADEX, available from Pharmacia; BIO-GEL P, available from Bio-Rad Laboratories) and debriding (DEBRISAN, available from J&J), or may comprise silica gel, zeolites, dextramines, alginate gel, starch, cross-linked agarose, etc. DEBRISAN appears similar to SEPHADEX G-25, and both are available commercially.

The beads are sealed into the chamber, but may become unevenly distributed during shipment. A hydrophilic filter 9

-43-

keeps the beads in the concentrator chamber 10. Immediately after the addition of whole blood, the cartridge may be gently rotated and counter-rotated a few times to evenly distribute the beads behind the lip 3.

5 After the platelet-rich plasma has entered the concentration chamber 10, the cartridge 1 is gently rotated to mix platelet-rich plasma with concentrator beads 2. For best results, it is important to keep the slurry in motion because of the tendency for protein to locally concentrate around each bead, retarding
10 absorption. After a few minutes (e.g., 1-10 min., preferably 3-5 min.), the concentration is complete. Note that concentration by approximately three times corresponds to fluid surrounding close-packed spheres; thus, three times concentration is optimal. Concentration of more than three times is possible, but to
15 achieve best results, a second concentration chamber should be used. Thus, concentration of nine times is possible for a three-chamber cartridge containing two concentration chambers.

20 When concentration is complete, the centrifuge increases its rate of rotation and the increased centrifugal forces cause concentrated platelet-rich plasma to pass through the hydrophobic annulus. Finally, the centrifuge stops, and concentrated platelet-rich plasma runs to the bottom under the force of gravity and is ready to be removed.

-44-

Finally, note that the concentrator cup 10 is shaped like an inverted funnel to vent the lower chamber to the upper chamber allowing displacement of air from the lower chamber by plasma.

C. Annular Chamber Plasma Separator and Dextranomer Bead in
5 Open Cell Hydrophobic Foam Concentrator

In Figure 3, similar to Figure 1 in many respects, the ready cartridge is shown in the right half and the path of the liquid is shown by the dashed arrows on the left half. The entire chamber is radially symmetric except the septum 5 for the whole
10 blood inlet. The receiving cup 6 holds blood until centrifugal force causes blood to pass through the filter 4. In the separation chamber, centrifugal force causes cells to pass above the lip 16 into an outer annular chamber and accumulate therein; continuing centrifugal force causes cells to migrate into the
15 outer annulus and platelet rich plasma to remain within the separation chamber. The parts are sized so that the packed cell (e.g., erythrocyte and leukocyte) layer is fully contained within the outer annular chamber, which retains the cells when the centrifuge stops. When the centrifuge stops, the platelet-rich
20 plasma flows into the concentrator chamber 10.

Concentration comes about when platelet-rich plasma contacts concentrator beads 2 in the concentration chamber 10. These beads are a molecular sieve used in chromatography (SEPHADEX,

-45-

available from Pharmacia; BIO-GEL P, available from Bio-Rad Laboratories) and debriding (DEBRISAN, available from J&J), or may comprise silica gel, zeolites, dextramines, alginate gel, starch, cross-linked agarose, etc. DEBRISAN appears similar to
5 SEPHADEX G-25, and both are available commercially.

As in Figure 1, the beads are held by static electric forces onto the surface of open cell hydrophobic foam 15. The open cell hydrophobic foam 15 is a honeycomb-like hydrophobic material.

After the platelet-rich plasma has entered the concentration
10 chamber 10, the cartridge 1 is gently rotated to mix platelet-rich plasma with concentrator (dextranomer) beads 2. As the fluid penetrates the open foam 15, the beads lose their affinity for the foam and begin to swell. For best results, it is important to keep the slurry in motion because of the tendency
15 for protein to locally concentrate around each bead, retarding absorption. After several minutes, the concentration is complete. Note that concentration by approximately three times corresponds to fluid surrounding close-packed spheres; thus, three times concentration is optimal. Concentration of more than
20 three times is possible, but to achieve best results, a second concentration chamber should be used. Thus, concentration of nine times is possible for a three-chamber cartridge containing two concentration chambers.

-46-

When concentration is complete, the centrifuge increases its rate of rotation and the increased centrifugal forces cause concentrated platelet-rich plasma to pass through the hydrophobic annulus 12. Sintered plastic 12 is made of POREX, comprised of small particles of plastic fused to create a porous structure. Fluids and small particulates can flow freely through the tortuous pathways defined by the spaces between the fused plastic particles when the material is wet. If the plastic is not specially formulated or treated, the sintered plastic will be hydrophobic and when in the dry state will afford resistance to influx of water, more greatly as the size of the pathways is reduced. This property of "entry pressure" allows the wetting of the surface of a hydrophobic porous wall without substantial entry of fluid into the material. At greater pressure, i.e., when centrifugal force is increased (e.g., at 1000-10,000 rpm or higher), this resistive force is overcome and fluid breaks through the barrier to enter and pass through the porous wall.

The centrifuge stops, and concentrated platelet-rich plasma runs to the bottom and is ready to be removed through filter 13 as in Fig 6.

Finally, note that the concentrator cup 10 is shaped like an inverted funnel to vent the lower chamber to the upper chamber allowing displacement of air from the lower chamber by plasma.

-47-

D. Fibrous Matrix Plasma Separator and Dextranomer-Impregnated Disc Concentrator

Figure 4 is similar to the previous figures in many respects. Like Figure 1, the ready cartridge is shown in the right half and the path of the liquid by the dashed arrows on the left half. The entire chamber is radially symmetric except the septum 5 (the whole blood inlet).

Separation is done by driving cells into a felt or foam mat 7. The parts are sized so that the packed cell (e.g., erythrocyte and leukocyte) layer is fully contained within the felt or foam filter, which retains the cells when the centrifuge stops.

In this design, even distribution of the dextranomer beads is assured by using plastic discs embossed with dextranomer beads. This can be done either with heat or solvent softening of the plastic followed by dipping in dextranomer beads or affixing beads to the plastic with adhesive. As the beads swell they detach from the discs. Separation of the concentrated platelet-rich plasma is done as in the above description of the present method of making concentrated platelet-rich plasma.

-48-

E. Method of Manufacture of the Disposable Cartridge

The various rigid plastic components comprising the cartridge are manufactured by injection molding or by pressure- or vacuum-forming, assembled and sealed by conventional methods (e.g., either by adhesives or welding). From Figures 1 through 4 it will be apparent to one skilled in the art of plastics fabrication that manufacture is relatively simple and inexpensive. The final assembled product is packaged, radiation sterilized and ready for distribution.

F. Plasma Concentrate, Platelet-rich plasma Concentrate and Tissue Sealant

The present invention also concerns a plasma concentrate, prepared by the present process. The present plasma concentrate may be platelet-rich, platelet-poor or platelet-free (i.e., the platelet concentration is not limited), but is preferably platelet-rich. The concentration of platelets can be controlled by the sedimentation rate, in accordance with known procedures and/or known platelet sedimentation behavior. Preferably, the plasma concentrate is autologous (administered to the patient from whom the whole blood was taken).

In one embodiment, the present plasma concentrate comprises platelets, from 5 to 400 mg/ml of fibrinogen, from 0.5 to 35 mg/ml of fibronectin, and a physiologically acceptable carrier

-49-

comprising water and physiologically acceptable inorganic and organic ions in a physiologically acceptable concentration. In a preferred embodiment, the plasma concentrate is prepared from whole blood, and platelets are concentrated at least one-and-one-half times relative to the concentration of platelets in unconcentrated plasma from the same whole blood.

The present plasma concentrate may further contain a compound selected from the group consisting of an antibiotic, a collagen fleece, collagenase, hyaluronic acid, a wound-healing factor, zinc ions in an amount effective to decrease coagulation time of said plasma, and a biologically acceptable dye. A preferred biologically acceptable dye is disulphine blue, contained in an amount sufficient to visibly detect the plasma concentrate during its application to tissue. In a preferred embodiment, the plasma concentrate further comprises a pharmaceutically active compound, particularly an antibiotic to reduce the probability of infection by extraneous microorganisms.

The present invention also concerns a tissue sealant, comprising the present plasma concentrate and an activator. Preferably, the plasma concentrate in the present tissue sealant is platelet-rich plasma concentrate.

In one embodiment of the present tissue sealant, the activator is a mixture of thrombin and calcium in amounts sufficient to permit gelation of the plasma concentrate. In a

-50-

further embodiment, the activator is autologous thrombin and platelets.

G. Apparatus for and Method of Mixing Two-Component Fibrin Sealant

5 Parallel syringes with the two components (e.g., concentrated platelet-rich plasma and calcium/thrombin solution) converge on a coaxial needle with mixing of the components at the application site, thus avoiding premature gelation; see Fig. 7. In the barrel of the syringe a cylindrical chamber 31 containing
10 thrombin and calcium solution is surrounded by an annular chamber 32 containing concentrated platelet-rich plasma. Plunger 30 causes the solutions to exit the apparatus through coaxial needles and mix at the tip 36. An optional sleeve 34 ensures proper mixing, and may be retracted to expose premature gel for
15 easy removal.

H. Dispenser/Activator for Platelet-Rich Plasma Concentrate

Most users of fibrin sealants have resorted to the use of bovine thrombin to induce gelation. In a preferred embodiment of the present invention, it is desirable to avoid excessive use of
20 bovine proteins in the final preparation. Thus, a method and apparatus employing a small amount of bovine or other thrombogenic enzyme to trigger the autocatalytic activation of

-51-

the coagulation process is envisioned. Fig. 8 shows a syringe 21 and applicator needle tip 23 separated by activator cartridge 22 through which platelet-rich plasma passes for activation by immobilized thrombin to convert fibrinogen to fibrin.

5 In a further optional embodiment, a needle designed to disrupt platelets may be used to apply a single-component concentrate (i.e., the concentrated plasma) without the use of thrombin. Fig. 8 shows a syringe 21 and applicator needle tip 23 separated by activator cartridge 22 through which platelet-rich
10 plasma passes for activation by, e.g., nylon wool. In this embodiment, calcium is added to the concentrate immediately prior to or during its application to tissue in need of sealing.

A porous membrane or other material with high exposed surface to void volume ratio can be derivitized with thrombin or
15 other thrombogenic or thrombin-like enzyme by known methods. The derivatized membrane is fitted between syringe and applicator needle.

In a separate but related embodiment, glass wool, nylon wool or collagen sponge or another material may be used in a similar
20 configuration to cause eversion of the platelets, known to initiate coagulation.

In a separate but related embodiment, concentrated plasma may be kept cold to prevent gelation. After application, the heat of the living tissue will initiate gelation.

-52-

A big problem with prior precipitation methods is that the fibrinogen must be reconstituted. This is difficult and may take hours, and only a relatively low concentration is achievable. Thus not only does the present invention allow more rapid availability, a surgeon can decide that "this one is a bleeder" and have sealant in 15 minutes. Previous technology requires that the surgeon order fibrin glue before the procedure begins.

The present concentrated platelet-rich plasma can be left in the device until needed, for hours if necessary, without spontaneous gelation.

Other autologous precipitation methods require that blood be drawn days in advance to allow time so that there is time for processing. Besides the inconvenience, this procedure allows the possibility of tracking errors and thus infection.

Generally, a proteolysis inhibitor such as aprotinin is added to the fibrinogen component of fibrin glues. (Academic opinion is actually divided on whether this is necessary; need has never been proven rigorously.) The present methods for producing concentrated PRP retain the natural inhibitors which are evidently lost in precipitation steps.

Fibrinogen from fresh plasma produces a mechanically superior gel (based on the general properties of proteins damaged by precipitation). Platelets (contained in the present plasma concentrate) are useful for wound healing (many references

-53-

available). Thus, an advantage of the present technology is that it provides better wound healing than conventional fibrin glues, which do not contain platelets.

Surgeons, particularly in the U.S., are likely to use bovine thrombin to "activate" the present tissue sealant. This is not a regulatory problem because bovine thrombin is available and is used routinely in surgery. However, the present invention provides a dispenser that activates prothrombin present in the concentrated platelet-rich plasma and/or that disrupt platelets so that clotting is initiated by one or more components in the concentrated plasma.

The present tissue sealant and autologous tissue sealant do more than merely seal the wound and adhere the damaged structures. In particular, the present autologous tissue sealant contains the patient's own living thrombocytes and wound healing factors, and thus, it nurtures the healing process. Other advantages of the present tissue sealant include the presence of attachment factors which (i) improve adhesion to damaged tissue and (ii) promote cellular infiltration. In the present autologous tissue sealant, which is made from the patient's own blood, important advantages include the ease, speed and convenience of preparation (it may be prepared in the operating room immediately before use). Furthermore, the optional absence of bovine thrombin in the present autologous tissue sealant

-54-

avoids immune system and foreign body reactions and eliminates disease transmission (i.e., from the bovine thrombin source).

I. Separator and Method for Separating Particulates from Suspensions

5 In a further aspect of the present invention, a separator and a general method for separating particulates from suspensions is envisioned. Separating particulates from biological fluids (such as blood and plasma) is a common problem in the laboratory. A disposable cartridge containing a separation chamber (i.e., the
10 first chamber in the apparatus used for preparing the present concentrated plasma) allows cellular and dense particle separation -- basically anything that moves in response to centrifugal force.

Thus, the present apparatus for separating particulates from
15 a liquid suspension comprises:

an inlet,

a first chamber in fluid communication with the inlet, containing a first separator for separating particulates from the liquid suspension, thus forming a particulate-free liquid, and

20 an outlet for withdrawing the liquid.

A "particulate-free liquid" is one which is substantially free of insoluble particles, preferably those insoluble particles having an average size above a pre-selected value (e.g., from 500

-55-

to 20 μ , or any value for which filters or other devices are available for removing such insoluble particles; e.g., 250 μ , 100 μ , 80 μ , 50 μ , etc.).

J. Concentrator and Method for Concentrating Macromolecule
5 Solutions

In an even further aspect of the present invention, a concentrator and method for concentrating macromolecule solutions based on the design of the concentrator (or second) chamber used in the apparatus for preparing the present concentrated plasma is
10 envisioned. SEPHADEX (trademark, Pharmacia), for example, is rarely used for concentrating macromolecule solutions. The device may not concentrate to dryness, but is useful for concentration of proteins (or any high molecular weight solution), preferably by a factor of three times or a multiple of
15 three times, depending on the number of concentrator chambers in the apparatus.

Thus, the present apparatus for concentrating a solution of a substance having a molecular weight greater than a predetermined value (i.e., macromolecule solution) comprises:

- 20 an inlet,
 a chamber in fluid communication with the inlet,
containing a concentrator for concentrating the solution and a separator for separating the solution from the concentrator, and

-56-

an outlet for withdrawing the concentrated solution.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limited thereof.

EXAMPLE 1

Using a cartridge as shown in either Figures 1, 2, 3 or 4, and following the procedure below, a concentrated platelet-rich plasma and tissue sealant can be prepared:

- I. Collect blood from patient using standard citrate anticoagulant.
- II. Inject a standard quantity of blood (e.g., 50cc) in to the blood fill port of the disposable cartridge. The cartridge is sealed making contamination impossible. It will be used in the operating room making tracking errors impossible.
- III. The operator then starts the microprocessor-controlled centrifuge by push-button. All remaining steps until removal of the concentrate are automatic.
- IV. (Optional step for designs incorporating free concentrator beads, Figure 2) The centrifuge rotates briefly clockwise then counterclockwise a few times at a speed sufficient to

-57-

evenly distribute the free dextranomer beads outside the concentrator cup lip.

V. The centrifuge spins at 3,000 rpm for one to ten minutes to separate platelet-rich plasma from cells. The whole blood
5 passes through a pore in the blood receiver chamber into the separation chamber.

VI. The centrifuge stops for 15-90 seconds. Under the force of gravity the platelet-rich plasma passes into the lower chamber, or concentrator chamber, (optionally by going
10 through a through a hydrophilic filter, appropriate when free beads are present in the concentrator chamber as in Figure 2).

VII. In the concentrator chamber platelet-rich plasma comes into contact with beads bonded by electrostatic force to an open
15 cell foam (cartridge of Figures 1 and 3), free concentrator beads (cartridge of Figure 2), or beads embossed onto discs (cartridge of Figure 4). Slow rotation of the centrifuge aids mixing. The porosity and hydrophobicity of the porous wall are such that the low centrifugal force generated is
20 not sufficient to overcome surface tension and the plasma remains within the compartment at the inner surface. The beads swell with water, ions and low molecular weight proteins (i.e., those proteins having a molecular weight below the cutoff value). The pore size of the beads is so

-58-

chosen that the proteins become concentrated in the space around the beads. The quantity of beads is chosen so that the platelet-rich plasma is concentrated the desired amount, e.g., 3X. When using DEBRISAN, approximately 2.6 gm is required per 10cc plasma to achieve 3-fold concentration. The protein concentrate contains the same ion concentrations as the unconcentrated plasma.

VIII. The rate of rotation is increased forcing the concentrated platelet-rich plasma out through the filter into the outer compartment.

IX. The centrifuge stops, and under the force of gravity concentrated platelet-rich plasma collects in the bottom. The concentrated platelet-rich plasma may be left for extended periods (hours) without spontaneous gelation.

X. Concentrated platelet-rich plasma is removed into a syringe.

XI. (Optional.) Thrombin/calcium solution is prepared separately.

XII. Using a variety of methods described in the literature for cryoprecipitated fibrinogen, the two solutions are combined and rapidly gel to form the TISSUE SEALANT.

XIII. (Optional; substitute for XI and XII.) Using a needle tip designed to disrupt platelets, a single-component concentrate may be applied without the use of bovine thrombin.

-59-

A comparison of the properties of the present tissue sealant and prior tissue sealant prepared from cryoprecipitated fibrin is shown in the Table below:

TABLE: Concentrations of Tissue Sealant Compositions

Blood Component	Precipitate Fibrin Glue	Autologous Concentrated PRP (3X)
Ions	per reconstitution solution	identical to patient plasma
Platelets	none	3X plasma concentration
Proteins	only proteins that cryoprecipitate; fibrinogen, factor XIII, fibronectin, albumin, plasminogen	all plasma proteins at normal (less than 5 kDa) or 3X normal (more than 5 kDa) concentrations
Albumin	10-25 mg/ml	100-165 mg/ml
Factor XIII	75 µg/ml	30-60 µg/ml
Fibrinogen	70-110 mg/ml	5-14 mg/ml
Fibronectin	2-9 mg/ml	0.5-1.2 mg/ml
Plasminogen	20-60 µg/ml	600 µg/ml
Aprotinin	1,500 KIU/ml	0 KIU/ml

-60-

The present tissue sealant also appears to show similar tensile strength to that of cryoprecipitated fibrin tissue sealant. The adhesive strength provided by the present tissue sealant is believed to be superior to that of cryoprecipitated fibrin tissue sealant because fibrinogen and other important proteins are not denatured by the present process.

A key advantage of the present invention is the presence of platelets. Adhesion of the platelets to collagen III fibrils leads to platelet aggregation, promoted by 5-hydroxytryptamine and epinephrine released from the platelets. Other growth and healing factors are released by the platelets. Platelet-derived growth factor is a mitogen for fibroblasts and smooth muscle cells. Platelet factors stimulate neovascularization, especially when the area is anoxic. Platelets also make fibrin more resistant to mechanical shear forces and to fibrinolysis.

It is possible that for certain applications lesser numbers of platelets may be desirable. The invention may be adjusted to achieve this outcome. Because of their low mass compared with other blood cells, a preponderance of platelets is preserved in the final products when conditions are chosen to minimally separate the bulk of said more massive cells from whole blood. For example, a 4-inch diameter cartridge, 1 inch in height and fitted with a sintered plastic wall as in Fig. 2 will separate most of the blood cells from 50cc of whole blood when centrifuged

-61-

for 3 minutes at 2,000 rpm. By increasing the speed to 5,000 rpm and the time to 15 minutes, most of the platelets are also sedimented through the porous walls to yield plasma depleted of platelets. Such platelet depleted plasma may be desirable if, for example, it were desired that the plasma or concentrate derived therefrom should be processed by filtration through a sterile submicron membrane to render the final product suitable for storage for an extended time (days).

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

45. See, for example, DelRossi, A. J., A. C. Cernaianu, R. A. Vertrees, C. J. Wacker, S. J. Fuller, J. Cilley Jr., and W. A. Baldino. "Platelet-rich plasma reduces postoperative blood loss after cardiopulmonary bypass." J Thorac Cardiovasc Surg 100 (2 1990): 281-6.

-62-

WHAT IS CLAIMED AS NEW AND IS DESIRED TO BE SECURED BY LETTERS
PATENT OF THE UNITED STATES IS:

1. A method of making concentrated plasma, comprising the steps of:

- 5 (a) separating plasma from whole blood;
- (b) contacting said plasma with a concentrator to provide said concentrated plasma; and
- (c) separating said concentrated plasma from said concentrator.

10 2. The method of Claim 1, wherein said separating step (a) comprises centrifugally contacting said whole blood with a separator which bars passage of red blood cells and white blood cells in the absence of an applied centrifugal force, and applying a centrifugal force effective to pass red blood cells

15 and white blood cells in said whole blood through said separator.

3. The method of Claim 2, wherein said separator is a mat, foam or porous plastic annulus.

20 4. The method of Claim 1, wherein said separating step (a) comprises centrifugally settling said whole blood into a chamber shaped and positioned such that gravity does not permit drainage

-63-

of said red blood cells and said white blood cells in the absence of an applied centrifugal force.

5 5. The method of Claim 1, wherein each of said whole blood and said plasma contains (i) platelets, (ii) high molecular weight substances or (iii) both platelets and high molecular weight compounds.

10 6. The method of Claim 1, wherein said concentrator comprises a material selected from the group consisting of a dextranomer, SEPHADEX, dextramine, polyacrylamide, BIO-GEL P, silica gel, a zeolite, DEBRISAN, cross-linked agarose, starch and alginate gel, either in free bead form or attached to a matrix.

7. The method of Claim 6, wherein said material is attached to a matrix, and said matrix comprises a mat, a foam or a disc.

15 8. The method of Claim 5, wherein said whole blood and said concentrator are present in amounts by volume providing a concentration in said concentrated plasma of said platelets, said higher molecular weight compounds or both said platelets and said higher molecular weight compounds of at least one-and-one-half times the concentration of said platelets, said higher molecular

-64-

weight compounds or both said platelets and said higher molecular weight compounds in said plasma.

9. The method of Claim 8, wherein said concentration is about three times.

5 10. The method of Claim 1, wherein said blood contains an amount of a coagulation inhibitor effective to inhibit coagulation of said blood.

10 11. The method of Claim 1, conducted in a sealed cartridge having an inlet, a first chamber containing a first separator for separating said plasma from said whole blood, a second chamber containing said concentrator and a second separator for separating said concentrated plasma from said concentrator, and an outlet for withdrawing said concentrated plasma.

15 12. The method of Claim 1, further comprising, after said separating step (c), repeating said contacting step (b) and said separating step (c).

13. An apparatus for concentrating plasma, comprising:
an inlet,

-65-

a first chamber in fluid communication with said inlet, containing a first separator for separating plasma from whole blood,

a second chamber in fluid communication with said first chamber, containing a concentrator for concentrating said plasma and a second separator for separating concentrated plasma from said concentrator, and

an outlet for withdrawing said concentrated plasma.

14. The apparatus of Claim 13, wherein said first separator comprises a mat, foam or porous plastic wall which bars passage of red blood cells and white blood cells in the absence of an applied centrifugal force, but through which red blood cells and white blood cells pass in response to an effective applied centrifugal force.

15. The apparatus of Claim 13, wherein said first separator comprises a chamber shaped and positioned such that gravity does not permit drainage of said red blood cells and said white blood cells in the absence of an applied centrifugal force.

16. The apparatus of Claim 13, wherein said concentrator has a molecular weight cutoff of from 2 to 100 kDa.

-66-

17. The apparatus of Claim 13, wherein said concentrator is selected from the group consisting of a dextranomer, SEPHADEX, dextramine, polyacrylamide, BIO-GEL P, silica gel, a zeolite, DEBRISAN, cross-linked agarose, starch and alginate gel, either
5 in free bead form or attached to a matrix.

18. The apparatus of Claim 13, wherein said concentrator is embedded in or attached to a matrix comprising a mat, a foam or a disc.

19. The apparatus of Claim 18, wherein said matrix
10 comprises a plurality of discs.

20. A plasma concentrate, prepared by the process of Claim
1.

21. The plasma concentrate of Claim 20, wherein said plasma concentrate is autologous.

15 22. A platelet-rich plasma concentrate, prepared by the process of Claim 5.

23. A plasma concentrate, comprising platelets, from 5 to 400 mg/ml of fibrinogen, from 0.5 to 35 mg/ml of fibronectin, and

-67-

a physiologically acceptable carrier comprising water and physiologically acceptable inorganic and organic ions in a physiologically acceptable concentration.

24. The plasma concentrate of Claim 23, prepared from whole
5 blood, wherein said platelets are present in said plasma concentrate in a concentration of at least one-and-one-half times the concentration of said platelets in unconcentrated plasma from said whole blood.

25. The plasma concentrate of Claim 23, further containing
10 a compound selected from the group consisting of an antibiotic, a collagen fleece, collagenase, hyaluronic acid, a wound-healing factor, zinc ions in an amount effective to decrease coagulation time of said plasma, and a biologically acceptable dye.

26. The plasma concentrate of Claim 25, further containing
15 disulphine blue in an amount sufficient to visibly detect said plasma concentrate during its application to tissue.

27. The plasma concentrate of Claim 23, further comprising a pharmaceutically active compound.

-68-

28. A tissue sealant, comprising the plasma concentrate of Claim 20 and an activator.

29. The tissue sealant of Claim 28, wherein said activator is a mixture of thrombin and calcium in amounts sufficient to permit gelation of said platelet-rich plasma concentrate.

30. The tissue sealant of Claim 28, wherein said activator is autologous thrombin and platelets.

31. A method of sealing tissue, comprising the steps of:

- (a) preparing a plasma concentrate according to the process of Claim 1,
- (b) activating said plasma concentrate to provide an activated plasma concentrate, and
- (c) applying said activated plasma concentrate to tissue in need of sealing.

32. The method of Claim 31, wherein said plasma concentrate is obtained from blood drawn from a patient, and said tissue in need of sealing is in or on said patient.

33. The method of Claim 31, wherein said activating comprises contacting said plasma concentrate with a mixture of

-69-

thrombin and calcium in amounts sufficient to gel said activated plasma concentrate.

34. A method of sealing tissue, comprising the steps of:

- 5 (a) preparing a plasma concentrate according to the process of Claim 1, said plasma concentrate containing prothrombin, platelets or both prothrombin and platelets,
- (b) activating said prothrombin, said platelets or both said prothrombin and said platelets to provide an activated plasma concentrate, and
- 10 (c) applying said activated plasma concentrate to tissue in need of sealing.

35. The method of Claim 34, wherein said activating comprises irradiating said platelets with ultrasound sufficiently to result in gelation of said activated plasma concentrate.

15 36. The method of Claim 34, wherein said activating comprises heating said plasma concentrate sufficiently to result in gelation of said activated plasma concentrate.

37. An apparatus for sealing tissue, comprising

a chamber for containing tissue sealant,

20 an applicator needle, and

-70-

an activator member located between said chamber and said applicator needle, in fluid communication with each of said chamber and said applicator needle.

38. The apparatus of Claim 37, wherein said activator
5 member is a porous membrane derivatized with thrombin.

39. The apparatus of Claim 38, wherein said porous membrane is further impregnated with calcium ions.

40. The apparatus of Claim 37, wherein said activator member is a fibrous material which causes eversion of platelets.

10 41. The apparatus of Claim 40, wherein said fibrous material is glass wool, nylon wool, collagen, or diatomaceous earth.

42. A method of concentrating a macromolecule solution, comprising:

15 (a) contacting said solution with a concentrator having a molecular weight cutoff of from 2 to 100 kDa to provide said concentrated solution; and

(b) separating said concentrated solution from said concentrator.

-71-

43. The method of Claim 42, wherein said concentrator comprises a material selected from the group consisting of a dextranomer, SEPHADEX, dextramine, polyacrylamide, BIO-GEL P, silica gel, a zeolite, DEBRISAN, cross-linked agarose, starch and
5 alginate gel, either in free bead form or attached to a matrix or embedded into a disc.

44. The method of Claim 42, wherein, prior to said contacting step (a), said concentrator is dry.

45. The method of Claim 42, wherein said solution is a
10 biological fluid.

46. A method for separating particulate components from a suspension thereof in a liquid, comprising:

(a) in a sealed container, centrifugally contacting a liquid suspension comprising said particulate components and said
15 liquid with a mat, foam or porous plastic wall which bars passage of said particulate components in the absence of an applied centrifugal force, but through which said particulate components pass in response to an effective applied centrifugal force, and

(b) removing said liquid from said mat, foam or porous
20 plastic wall.

-72-

47. The method of Claim 46, further comprising, before said separating step (a), the step of placing said liquid suspension in said sealed container.

48. The method of Claim 46, wherein said particulate
5 components are biological cells or fragments thereof.

49. The method of Claim 48, wherein said particulate components are biological cells, and said biological cells are red blood cells and white blood cells.

50. A method for separating particulate components from and
10 for concentrating a liquid suspension containing a solution of one or more high molecular weight compounds and said particulate components, comprising:

(a) in a sealed container, centrifugally contacting
said liquid suspension with a porous membrane, a porous foam or a
15 mat which permits passage of said particulate components under an applied centrifugal force, but which does not permit passage of said particulate components in the absence of said applied centrifugal force, to separate said particulate components from said solution,

20 (b) concentrating said solution according to the process of Claim 42 to prepare a concentrated solution, and

-73-

(c) removing said concentrated solution from said sealed container.

51. An apparatus for separating particulate components from a liquid suspension, comprising:

5 an inlet,

a first chamber in fluid communication with said inlet, containing a first separator for separating particulates from said liquid suspension, thus forming a liquid, and an outlet for withdrawing said liquid.

10 52. An apparatus for concentrating a macromolecule solution, comprising:

an inlet,

a chamber in fluid communication with said inlet, containing a concentrator for concentrating said solution and a separator for separating said solution from said concentrator, and

15 an outlet for withdrawing said solution.

1 / 9

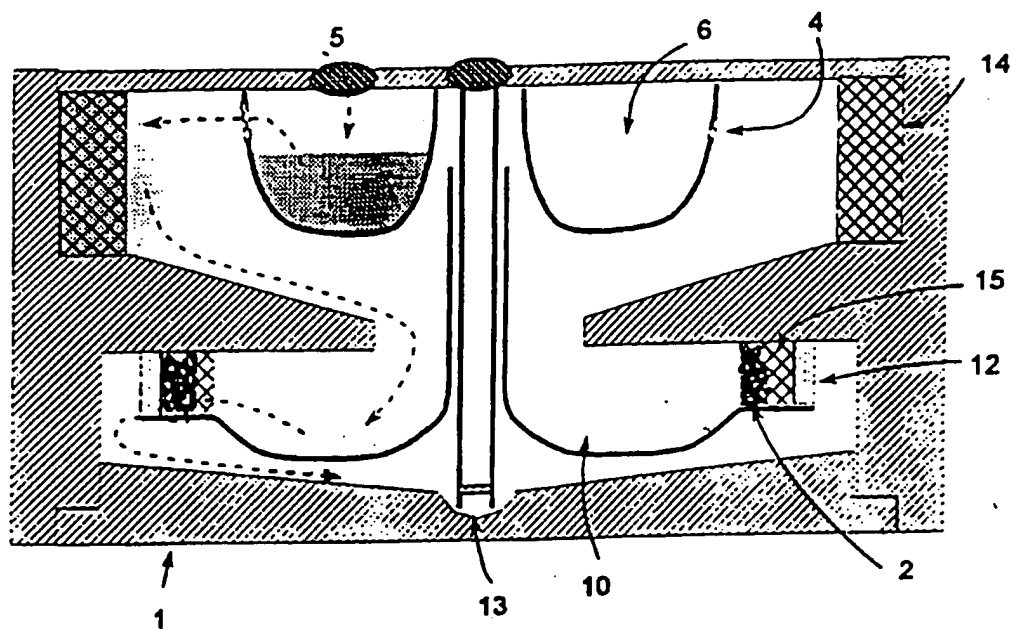


Figure 1.

2 / 9

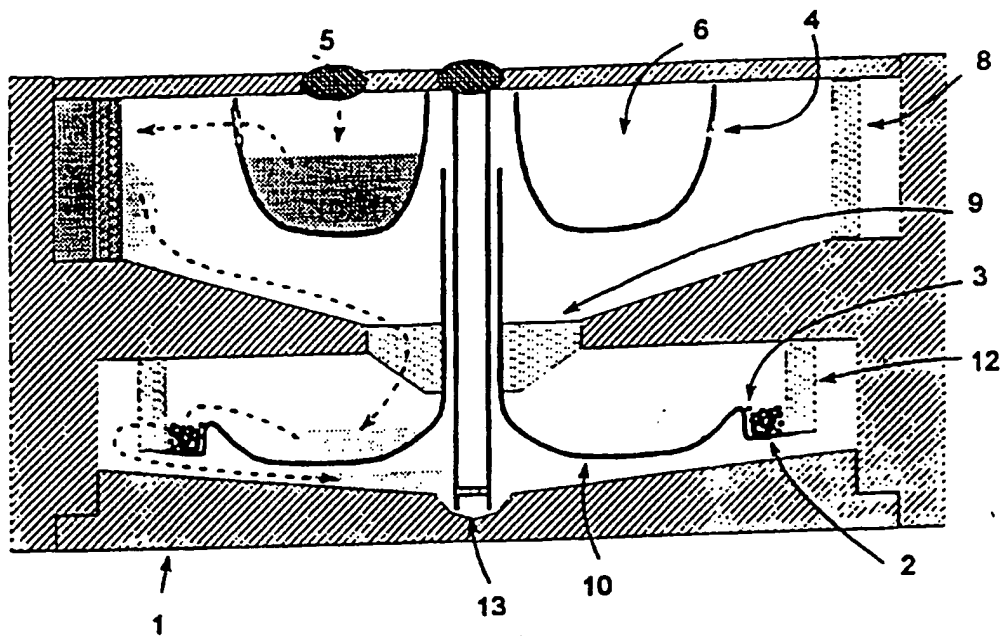


Figure 2.

3 / 9

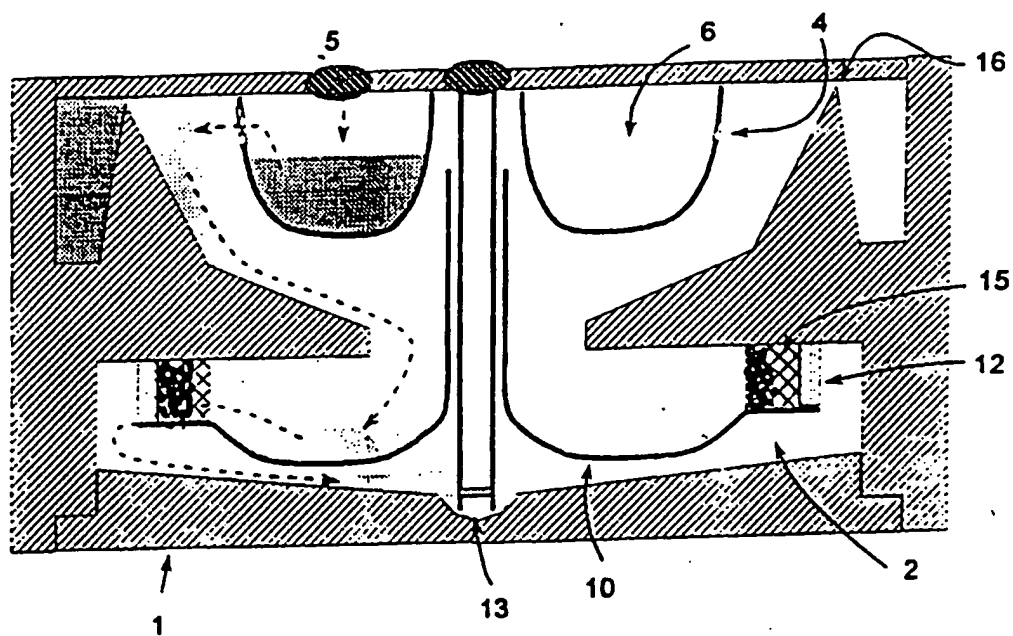


Figure 3.

4 / 9

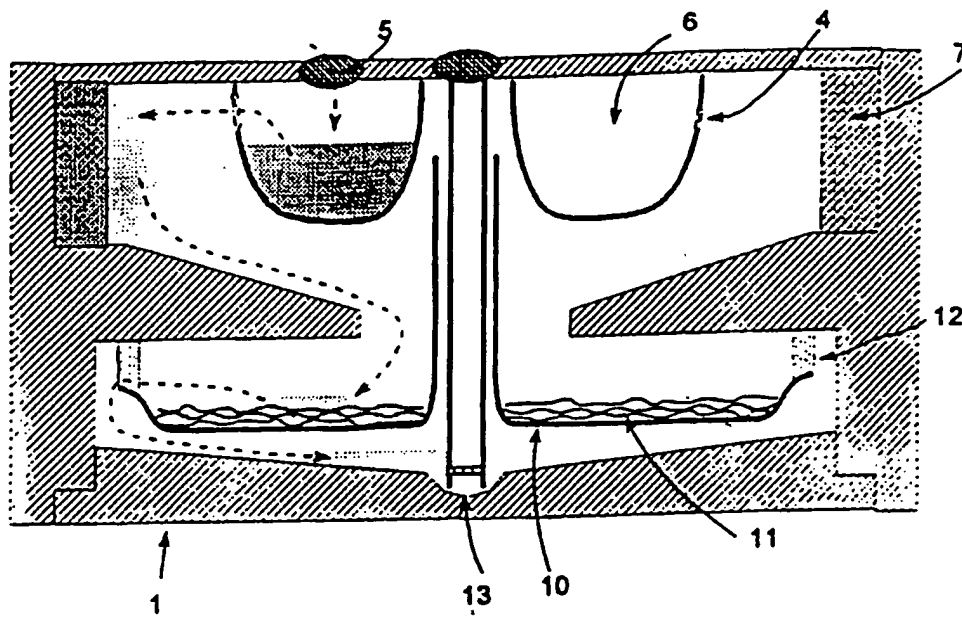


Figure 4.

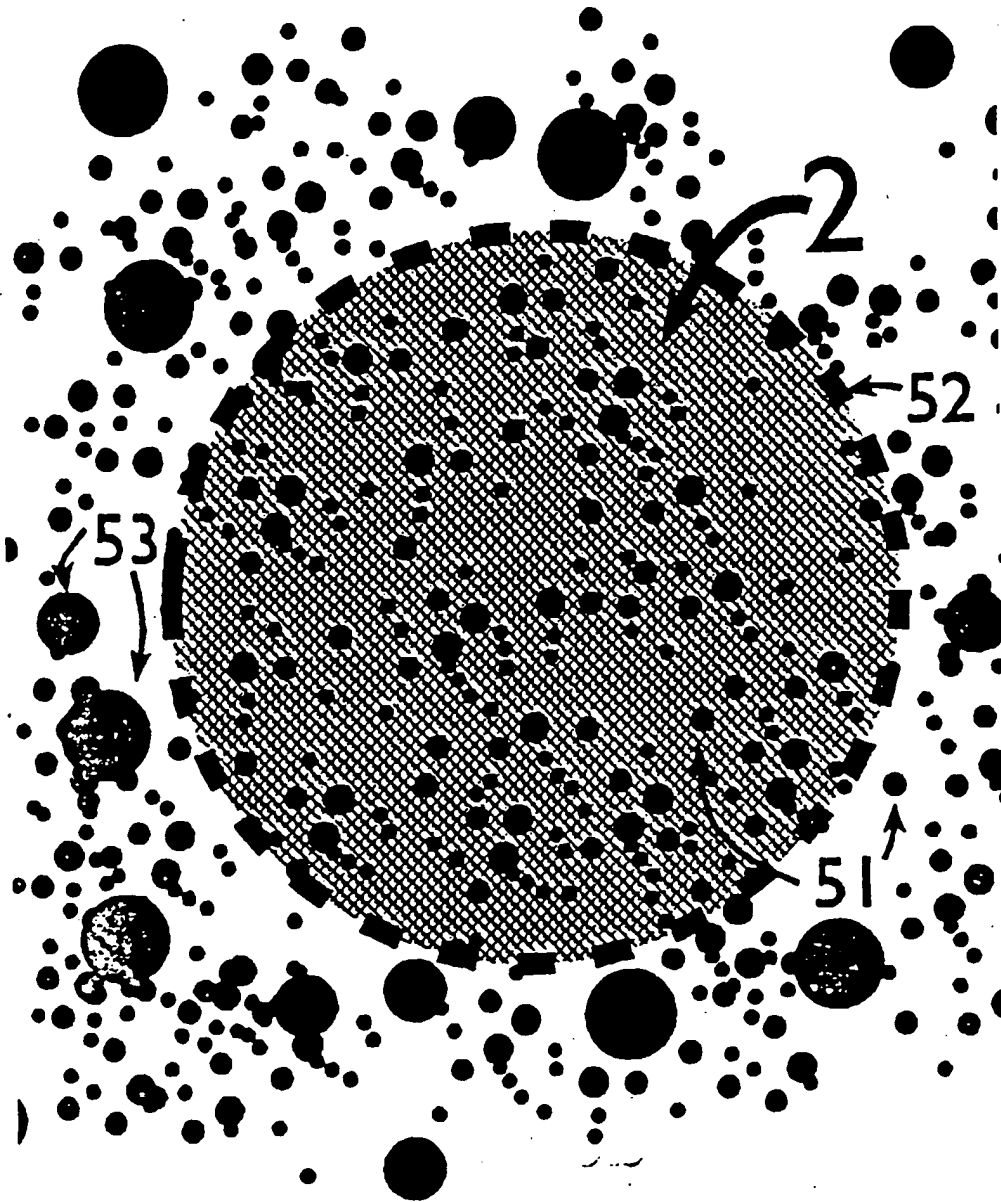


FIGURE 5

6 / 9

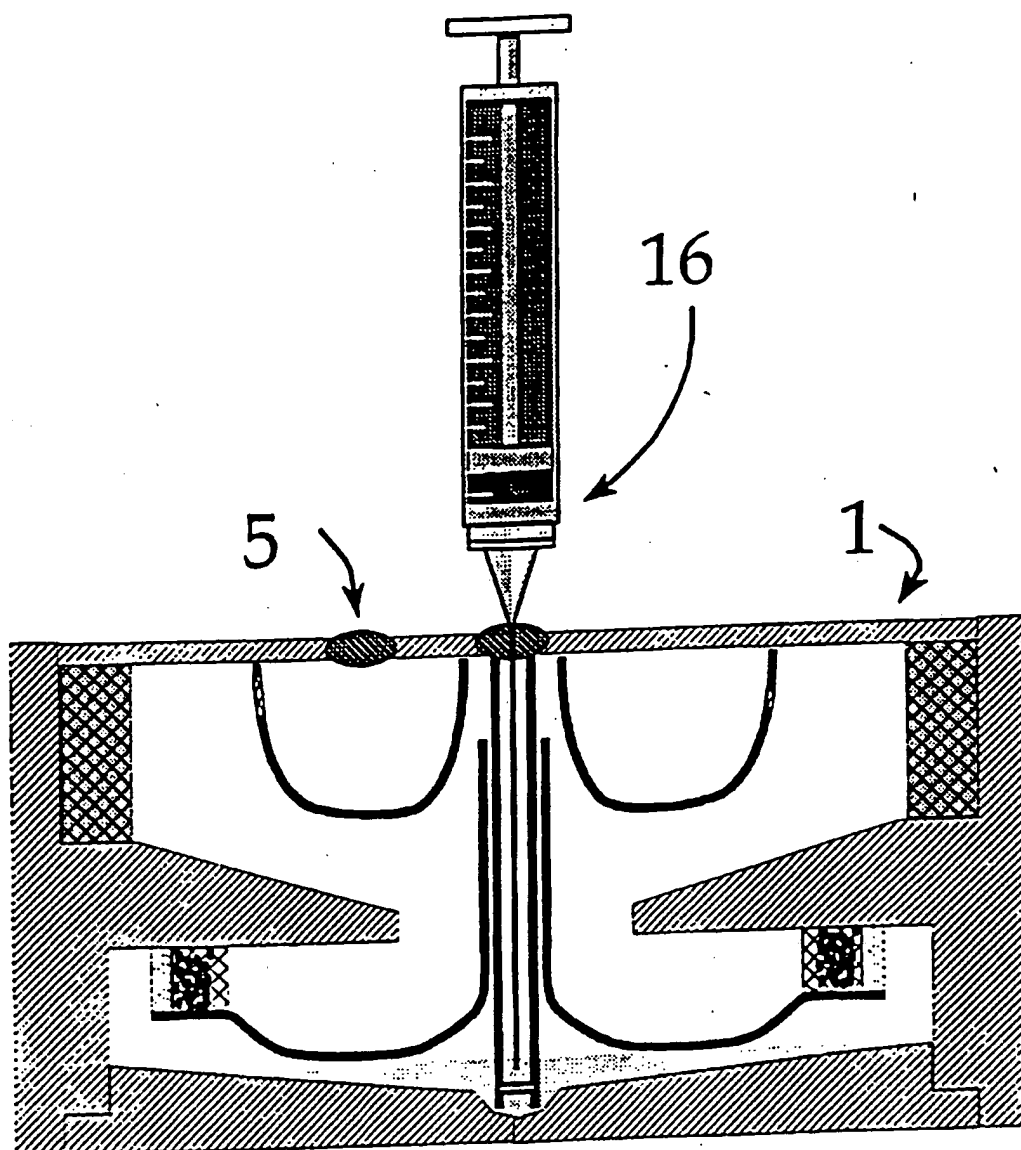


Figure 6.

7 / 9

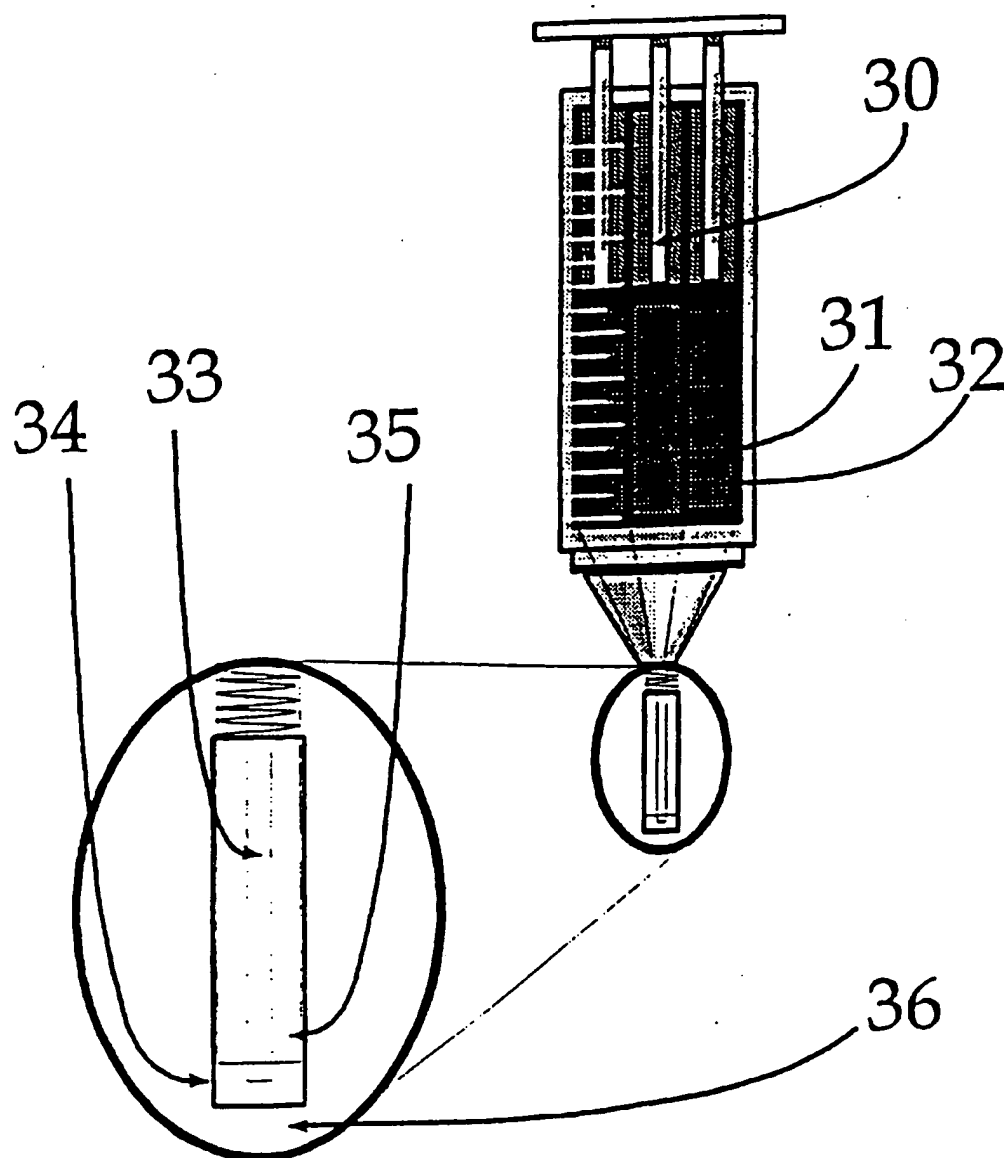


Figure 7.

8 / 9

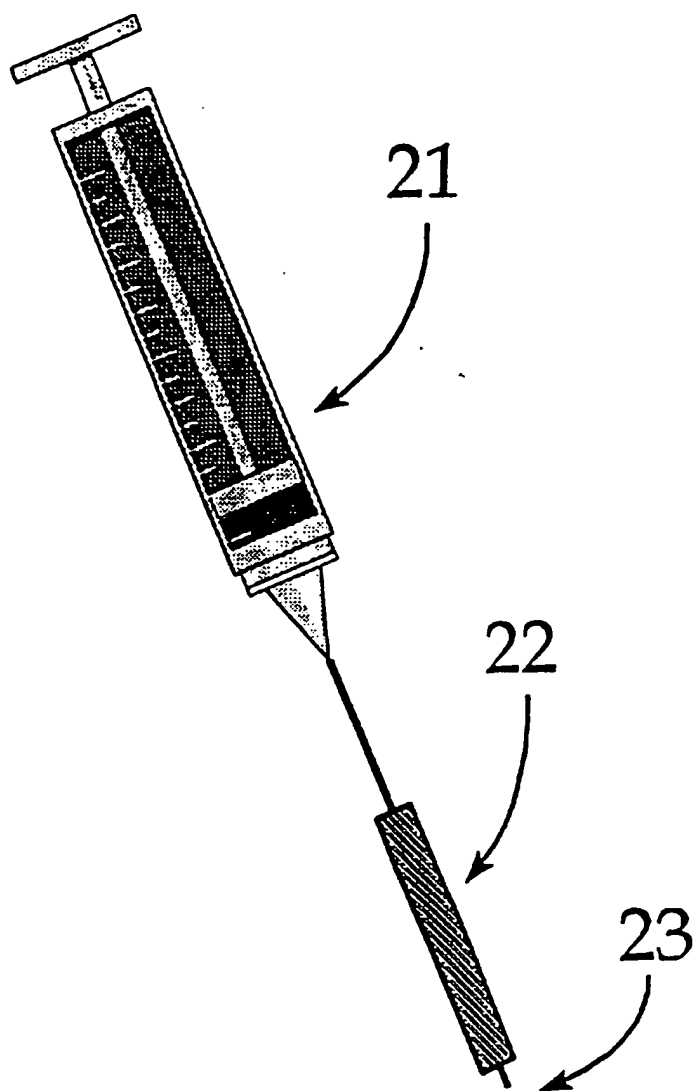


Figure 8.

9 / 9

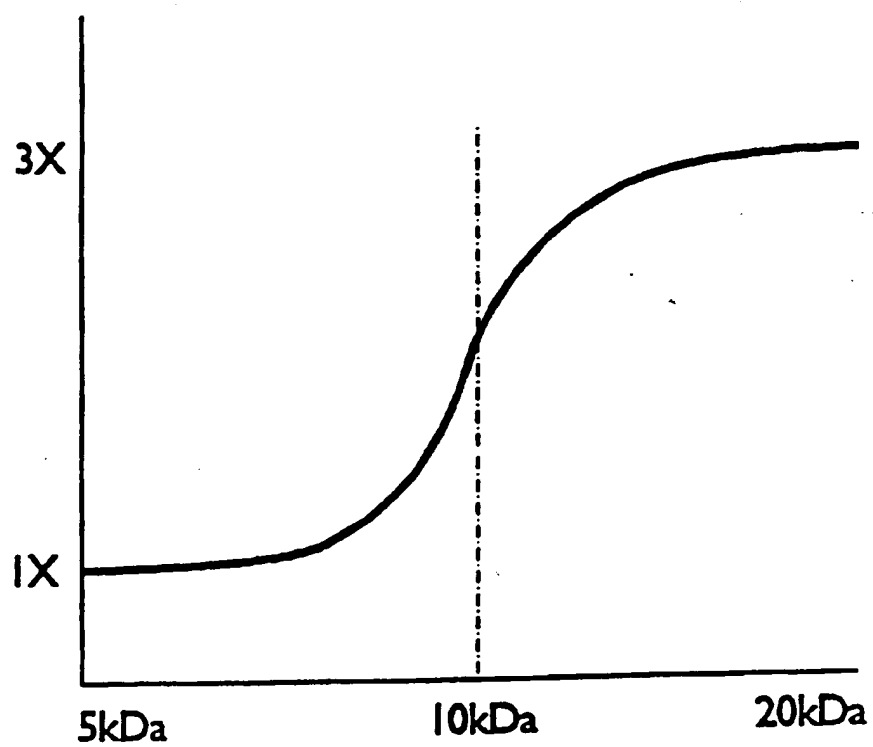


FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/15171

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/75, 14/745; A61K 9/10; B01D 15/00, 17/025, 17/038

US CL : 530/380, 381, 382, 829, 830; 422/255, 258; 424/484, 491; 602/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/380, 381, 382, 829, 830; 422/255, 258; 424/484, 491; 602/50

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, EMBASE, MEDLINE, DERWENT

search terms: plasma concentrat?, tissue sealant? adhesiv? glue?, platelet?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Vox Sanguinis, Volume 68, issued February 1995, Boomgaard et al, "Pooled Platelet Concentrates Prepared by the Platelet-Rich Plasma Method and Filtered with Three Different Filters and Stored for 8 Days", pages 82-89, see page 82, abstract, lines 1-18.	20-24
X --- Y	US, A, 4,322,298 (PERSIDSKY) 30 March 1982, see the abstract, lines 1-22, column 2, lines 64-68 and column 8, lines 26-44.	1-2, 4-5, 8, 10-11, 13, 15, 20-24 ----- 3, 6-7, 9, 12, 14, 16-19, 25-36

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A		document defining the general state of the art which is not considered to be of particular relevance
* E		earlier document published on or after the international filing date
* L		document which may throw doubts on priority claims or which is cited to establish the publication date of another citation or other special reason (as specified)
* O		document referring to an oral disclosure, use, exhibition or other means
* P		document published prior to the international filing date but later than the priority date claimed
	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
	* Z	document member of the same patent family

Date of the actual completion of the international search

31 JANUARY 1996

Date of mailing of the international search report

25 MAR 1996

 Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

NANCY DEGEN

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/15171

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,152,905 (PALL ET AL) 06 October 1992, see the abstract, lines 1-5, column 2, lines 28-35, column 6, lines 14-68 through column 7, lines 1-9 and lines 32-53 and column 8, lines 20-45.	1-36
Y	US, A, 5,226,877 (EPSTEIN) 13 July 1993, see column 4, lines 60-68 through column 5, lines 1-3, and lines 44-56 and column 10, lines 45-68 through column 14, lines 1-58.	1-41
A	US, A, 4,424,132 (IRIGUCHI) 03 January 1984.	1-41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/15171

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-41

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/15171

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Group I, claims 1-27, drawn to a plasma concentrate, a method of making the concentrate and an apparatus for the making of the concentrate.

Group II, claims 28-41, drawn to a tissue sealant, a method of making the tissue sealant, and an apparatus for using the tissue sealant.

Group III, claims 42-52, drawn to a method and apparatus for concentrating a solution and a method and apparatus for separating particulate components from a suspension.

The inventions listed as Groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Plasma concentrates and tissue sealants may be made by significantly different processes and apparatuses. The concentrator and separator may be used to concentrate and separate other types of solutions, including non-biological solutions.